

nature

14 September 1978

Why go to the British Association?

IN many ways the annual meeting of the British Association for the Advancement of Science, just concluded in Bath, was quite a success. There was an excellent turnout of members of the young scientists' wing of the organisation—several hundred students in the 11-to-18-year-old category were regular attenders at the meeting and seemed both to enjoy themselves and learn a lot. Audiences were supplemented by the usual senior contingent of enthusiasts for science, some with a professional interest in science, others keen amateurs. A wide variety of lectures was available, including, as is customary, sections on geography, sociology and economics. Symposia on broad topics such as national food requirements, science and technology for development and decision-making in science drew reasonably good crowds. The only thing missing was a reasonable turnout of the research scientists—academic, industrial and governmental—whose work the association attempts to portray.

Certainly there were nearly three hundred speakers in the four days of the meeting, and most of these were actual researchers themselves. But many of these stayed long enough to deliver their wares and no longer. The number of those actively involved in research (outside the officers of the sections) who gave the meeting four, three or even two days of their time was exceedingly small.

There are some very sound reasons for this: the meeting comes in the midst of university vacations; there are other more specialised conferences that have to be attended; there is a limit to the number of conferences any one scientist can be expected to attend, and (a circular and slightly less sound argument) there are not enough like-minded people around to warrant staying any length of time.

Of course the meeting is not a high-powered affair and there is no obvious immediate research benefit

from attendance, but are the channels by which scientist can speak to scientist across the disciplines so good elsewhere that the British Association can be ignored? For one of the most widely remarked features of science is that significant advances frequently occur when people from one discipline discover intellectual sustenance in another, maybe remote discipline. The chance to make new contacts over a period of a few days seems just the sort of 'removal of those disadvantages that impede scientific progress' which the founders of the association aimed at.

But there is another more selfless reason why research workers should attend the annual meeting more consistently—the good effect they can have on the enthusiastic young contingent. Whilst it is not necessary that every lecture delivered to the association be comprehensible to a 16-year-old, many of these young scientists would value relaxed conversations with people whom they obviously respect. Such conversations would help them understand just what it is like to be a scientist and which particular fields of study might appeal to them. The profession of research scientist is not easily understood from outside and is subject to many misapprehensions; evenings of chat in coffee bars are probably as good a way as any of dispelling illusions and clarifying ambitions.

If there was one other disappointment in the attendance at Bath it was the poor, almost negligible turnout of those who are involved in big decisions which can have a scientific content. One would have thought that many politicians, top civil servants and leading industrialists would use such an occasion to keep well informed and to sound out their constituencies. Not so. This is done in comparable assemblies in some other countries, but not in Britain. The result is a narrow-mindedness and compartmentalisation that seems particularly severe in British public life.

A. J. V. Gale 1895-1978

ARTHUR GALE, joint Editor of *Nature* from 1939 to 1961 and associated with the journal since 1920, died on 4 September 1978.

Educated at Selwyn College Cambridge, he was involved in military service for most of the First World War, and was then persuaded by Sir Richard Gregory, recently appointed Editor, to join *Nature* as Assistant Editor. In 1939, on Gregory's retirement, the editorship passed into the joint hands of Mr Gale and Mr L. J. F. Brimble, who had come to *Nature* in 1931. Brimble continued after Gale's retirement to edit the journal single-handed until his death in 1965.

In order that Gregory and later Brimble should lead a lively public life, attending conferences, meeting scientists, championing causes, it was necessary that someone of scrupulous habits should be back in the

office ensuring that the journal appeared on time each week, and that the appropriate bread and butter of *Nature* was attended to. This Gale did to perfection. He knew exactly what *Nature* was about, and he understood the science well, but his responsibility was not to comment or interpret but to ensure that other people were free to do so. He was essentially the servant of *Nature* and thereby of the whole scientific community.

He claimed that he only ever wrote two leaders, yet paradoxically they were at a most momentous time for science's relation with society: the two weeks following Hiroshima and Nagasaki. His calm and sensible words at that time made one regret that he did not write more often.

He married Gwendoline Veysey in 1929 and to her and to his son and daughter we send our sympathy.



Biologists urge US endowment for conservation

One in five animal species may be extinct by the year 2000, writes **John Douglas**

AMID dire predictions about the future of tropical forests and endangered species, an international group of biologists has called on the United States government to establish a national endowment, similar to the national endowment for the arts, "to support original research, both theoretical and applied, in the area of conservation biology". The conference also called on governments and private groups of all developed nations to offer their support to establish and protect "some remnants of natural habitat in tropical countries". Both resolutions were adopted by acclamation at the end of a four-day conference on conservation biology at the University of California, San Diego, last week.

In recommending establishment of the new research endowment, the conference members cited the present lack of any appropriate US agency to fund and coordinate research in environmental management, wildlife biology and conservation. "In particular, we need an agency to fund research relevant to the stewardship of the Earth's remaining natural diversity". Specifically, the resolution calls for increased support for breeding programmes to help save endangered species, research on the ecology of endangered habitats, and an inter-disciplinary approach to the biology of conservation, environment, and animal populations.

To help preserve remaining tropical habitats, the conference again called on the US government to take the lead by launching a major programme "to aid underdeveloped nations financially in establishing and protecting national parks and biological reserves". Otherwise, the resolution concluded, destruction of tropical forests may seriously affect climatic stability, soil erosion, water quality and economic development, "generally impoverishing human existence".

The plight of tropical forests was detailed in a paper by T. C. Whitmore of the University of Oxford. "The second half of the 20th century will stand in history as the brief period during which man reduced the area of the world's richest and most complex ecosystems to about one-third of their potential area", he said. "An onslaught has developed on tropical moist forests which is predicted to continue to accelerate to reduce them to scattered fragments by 2000 AD."

Between now and the turn of the century, the demand for tropical hardwood log production is expected to triple, said Dr Whitmore. That will

require cutting 556 million hectares of forest, leaving only 30% (480 million hectares) of the total area under virgin conditions. Furthermore, the process of using bulldozers to retrieve the logs may compact as much as 70% of the soil in affected areas, hindering forest regeneration. "The prospect is frightening" he concluded.

Even if large national parks and game preserves are established, however, the loss of the great tropical forests will halt the further evolution of most tropical vertebrate species and drive many animals into extinction, according to papers delivered by Michael Soule and Bruce Wilcox of the University of California, San Diego. By using species data from island habitats the authors estimated the minimum area required for various animals to continue speciation. Even the largest of today's national parks are too small by an order of magnitude to foster continued evolution of large mammals, they conclude.

As a result of inbreeding and environmental accidents, Soule and Wilcox predict that the animals trapped in reserves too small for their continued evolution will begin to die out. Taking 19 East African national parks

as an example, the researchers conclude that 11% of the large animal species will have become extinct in 50 years and 44% will be gone in 500 years, unless new management techniques are introduced. If present trends persist worldwide, only about 1% of the Earth's surface will remain in a preserved condition by the turn of the century, which would eventually lead to the extinction of perhaps 3.5 million of the known 5.0 million terrestrial animal species. One million may disappear just by the year 2000. "The ark is sinking" says Wilcox.

Several speakers addressed the question of how large a population of animals must be in order to avoid eventual extinction, and what breeding practices might be adopted to encourage the vitality of a species. Ian R. Franklin of the Genetics Research Laboratories (CSIRO), North Ryde, New South Wales, estimated that genetic considerations alone would require an "effective population" of at least 500 individuals. (Since not all members of a group of animals contribute equally to the gene pool of the next generation, the number of genetically "effective" individuals ranges between roughly half and three-quarters of the total population.) Below the minimum population, Franklin says, the random loss of desirable traits,

Animal behaviour



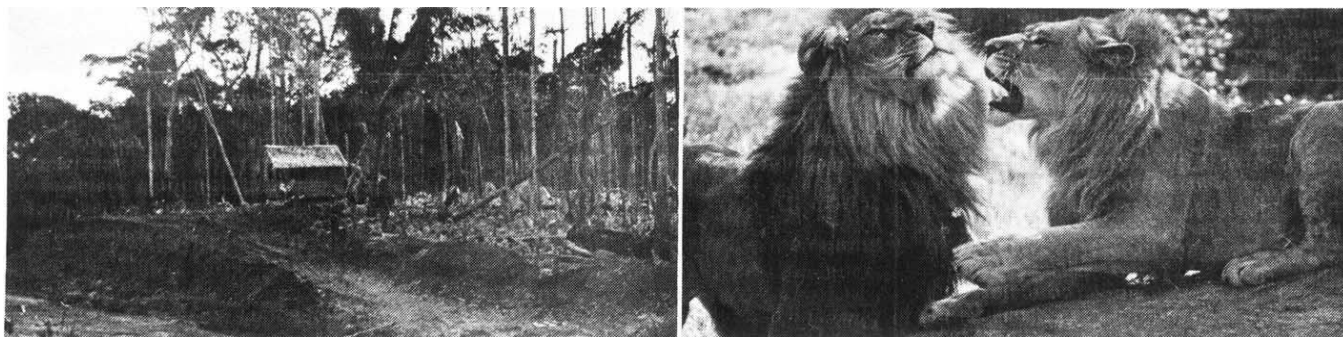
KENNETH MELLANBY

I WAS recently a member of a panel on a radio programme which discussed the topic "Do animals have rights?" The panel included representatives of animal protection societies and a scientist involved in research. The chairperson was a well-known journalist and broadcaster. We had a live audience of about 200 and

the programme, with short breaks for advertisements, went on for an hour and a half.

The programme started with the chairperson asking the members of the panel to state, briefly, their views on animal's rights. All agreed that animals should not be made to suffer unnecessarily, but disagreed as to what suffering meant, and on obvious subjects like vivisection, hunting and factory farming. I thought that we should concentrate on man's responsibilities rather than on animal's rights. Domestic animals, cattle and sheep, and pets such as dogs and cats only exist because man breeds them, and controls the numbers which are born. Without man, most breeds would soon become extinct, though in some areas cats have escaped and become pests, endangering the native fauna. As man has created domestic animals, he has the responsibility for providing conditions where they do not suffer—even conditions where we can reasonably imagine that they enjoy life. But if man disappeared from the world, so would most domestic animals.

The situation regarding wildlife is different. Wild animals, for the most



Left: trans-Ama-zonia highway and the jungle; right: maned lions each worth half-a-million dollars as Kenyan tourist attractions

called "genetic drift" will make a species unfit for survival.

Much of the problem of establishing and maintaining large reserves is economic: the need is greatest in areas encompassed by the world's poorest nations. William G. Conway of the New York Zoological Society concludes "The preservation of the majority of the animals that man finds attractive probably cannot be sustained on any provable economic basis". However, some nations, he says, have successfully used their preserves to bring in hard currency through tourism. In Kenya's Amboseli National Park, for example, a single maned lion is estimated to be worth \$515,000 as a tourist attraction, compared to only \$1,150 for commercial purposes as a skin.

For those animals that cannot be preserved in the wild, Conway suggests the limited use of zoos as "gene banks"

until future generations can be re-introduced into the wild. With careful mating, the number of individuals needed might be as small as 50 to 100 animals, he says, but even then half their genetic diversity would be lost and only 100 species could thus be maintained if half of all the zoo capacity in the United States were marshalled to the task.

Another way to preserve species by making some of them more profitable to indigenous people was discussed by Malcolm Coe of Oxford. The oryx, for example, is better adapted to life in Africa than cattle, he said, and some domesticated herds of eland, springbok and greater Kudu have been established. Another advantage of using such wild ungulates for food comes from their high proportion of lean meat — 43% compared to 23% of the carcass of cattle raised under the same

conditions. Even crocodiles might be raised as a valuable cash crop, since their hides bring a high price and they grow much faster in captivity.

The generally sombre message of the conference was phrased in particularly vivid terms by Thomas E. Lovejoy of the World Wildlife Fund. Between 50% and 85% of all species on Earth have yet to be named, he said, and a major fraction of these may pass entirely out of existence still unrecognised. They will be replaced by the quickly evolving, highly productive species that can coexist with man, often as pests. "Cockroaches and Norway rats will loom larger in 21st century bestiariums," says Lovejoy, "as will crabgrass and dandelions in the floras".

Bison trouble

BYELORUSSIA has been facing a somewhat unusual hazard of conservation at the—literally—grass-roots level. The primaevial Bielaviezskaja forest is a reserve for, among other species, Europe's last remaining bison. Virtually exterminated during the war, the bison herd is now multiplying in a most satisfactory manner, and it is hoped shortly to remove the bison from the Red Book of endangered species.

In the meantime, however, bison can occasionally cause problems — as certain collective-farmers found this summer. On the "Komintern" collective farm near Barysau, one particular bison claimed as its range a large hayfield, chasing the only farmer who dared approach it up a tall tree.

Denied more "radical means" of evicting it, the Farm Management Board decided to leave the field to the bison — whereupon several other bison moved in. To date no information is forthcoming as to whether the bison have been dislodged.

This is not the first time that forest bison have caused disruption of local life; a few years ago, at Hajdowka on the Polish side of the forest, one of the local bison went to sleep on the railway lines, following this exploit by a visit to the local school.

Vera Rich

part, only continue to exist where man has left conditions—habitats—suitable for them. As a conservationist I think that we should do more to preserve and develop such habitats, even though this conflicts with the needs of agriculture to produce food for the growing human population.

Although the producer of this radio programme had tried to provide a balanced audience, and had invited farmers, those engaged in animal experiments, and sportsmen to participate, these groups were greatly outnumbered by those who considered themselves to be animal lovers. There were colourful and hirsute young men and drab girls wearing T-shirts proclaiming them to be hunt saboteurs. We had anti-vivisectionists of all persuasions, vegetarians, some milk drinkers, some vegans, and some who seemed mainly to wish to oppose the politics of the establishment. Although there were good questions and sensible comments from the floor, the majority of the audience had not come to listen or to discuss. Their purpose was to stop those with whom they disagreed from speaking.

Thus when one panel member tried

to give the correct breakdown of the number of animal experiments in Britain, showing that these were mostly for genuine medical tests and not just to swell the profits of cosmetic manufacturers, a section of the audience, largely consisting of remarkably unattractive women resembling those who knitted when the guillotine functioned in revolutionary France, bayed "Lies, lies, lies, sit down, we won't listen to your lies". When I was asked about the policy of culling red deer in the Scottish Highlands, and said this was necessary to keep the population healthy, as otherwise, as wolves and other predators which controlled numbers were no longer present, the deer would increase until many died from starvation and disease, someone shouted: "Why not cull human beings?" The noise made by the audience was quite alarming, and made the interruptions heard at question time in the British parliament sound almost human.

I am afraid that my main reaction to the evening's entertainment was to wonder why those who profess to love animals hate their own species so much!

Science at the UN: coordination or chaos?

Peter Collins reports on the likely demise of the UN's only scientific advisory body, 'ACAST's. Who will replace it?

"**A**T least we should be represented at our own funeral" remarked the chairman of ACAST's recent meeting in Geneva. For this, the United Nations' Advisory Committee on the Application of Science and Technology, is almost certainly one of the bodies likely to disappear in the restructuring to which its parent body, ECOSOC (the Economic and Social Council) is now being submitted. In this process ACAST has no say, and may find few friends to speak up on its behalf: government delegates as a whole, and especially those of the developing countries who hold a voting majority, do not like independent committees. Be that as it may, the abolition of ACAST at the present juncture would be most unfortunate. Its recent meeting was one of the liveliest and most useful since its foundation, and its discussions had an air of realism and urgency often lacking in past. This was perhaps due to the presence of several new members of this individually selected body, invited to replace some of those who had given it the reputation of representing the scientific establishment, with few original ideas and, at times, little apparent awareness of the trends and possibilities of modern science and technology.

In search of harmony

One of the major subjects discussed was the matter of policy for science and technology within the UN system itself. ECOSOC went on record some time ago to the effect that "the planning of activities in the various organisations of the United Nations should be harmonised and gradually integrated into a United Nations science and technology policy".

However, the idea of a centrally-controlled, general policy for science and technology within the system is widely considered to be impracticable, even if indeed it is desirable. What is seen as a reasonable objective is the harmonisation of policies within and between the various parts of the system. Some decisions on this subject are expected to form part of the Programme of Action for UNCSTD next year, and some sort of closer coordination in this field is also seen as essential for the evolution of the "new international economic order", in which the whole UN system and its member governments are now involved.

When it came to the means of bringing about such harmonisation, ACAST considered three main possibilities. The

first of these was creation of a new agency charged with the overall coordination of science and technology for the entire system. ACAST commented that "experience in the United Nations (as well as with almost all organisations) is that new agencies become concerned not only with their own survival but also with increasing their influence and activities."

The second possibility, the suggestion of setting up an organisation for science and technology equivalent to the UN Environment Programme, was looked on somewhat more favourably. But to be effective it would sooner or later require, as has UNEP, a fund, and it is unlikely that member governments would agree to the creation of yet another large fund with all its administrative costs and the need for constant checks on the validity of the operations in which it might become involved. In any event, such a programme would have to rely on inputs from the rest of the system, and would need to have sufficient political or other support "to ensure consistency among the various agencies objectives and strategies through its decisions regarding funding and through coordination".

Much the most attractive, at least under present circumstances, would appear to be the third possibility, defined as a Centre for Concerted Action and Coordination on Science and Technology. Its purpose would be to systemise and synthesise information on all science and technology programmes and strategies throughout the system; to keep the Secretary-General advised of what was going on and what were the objectives and sub-goals of the other organisations within the system, relating these to the overall objectives; and to maintain contacts with the scientific and technical community, including intergovernmental and non-governmental organisations.

Questions involving research could be referred to other organisations within or outside the system, or, suggest ACAST, "to a committee of no more than twelve top-level experts or to such *ad hoc* advisory committees or working groups as might be necessary". Such a centre could create the setting for some sort of planned policy-making for science and technology within the system, besides providing a mechanism for coordination and harmonisation. Because it need have only a small staff, it could be flexible and could respond quickly to changing situations and in

providing advice to the Secretary-General. At the same time, it would compete with no existing organisation for either resources or authority.

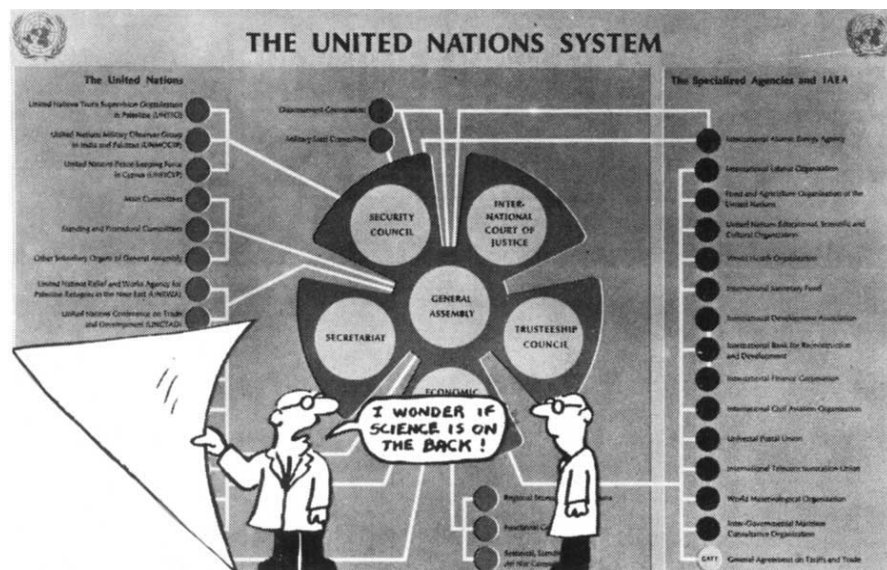
If set up at the present juncture, such a centre could be designed to allow for the present restructuring of the various organs involved in ECOSOC and in the central control of the UN itself. Nonetheless, it would still have to face the problem raised by the specialised agencies which still seem set against any form of coordination, let alone integration, as was also made clear at the ACAST meeting.

A message for UNCSTD

From the immediate point of view of the scientific community outside the UN, the most interesting discussions concerned the meeting to be held in Vienna in the week immediately preceding UNCSTD, 13-17 August, 1979. Known hitherto as "Forum A" (to distinguish it from "Forum B", the non-governmental event being held parallel with UNCSTD and organised by the Austrians themselves), this is now the responsibility of ACAST, and will probably be called the "ACAST Colloquium on Science, Technology and Society". It will be confined to some 200 invited scientists, technologists and other persons, including governmental representatives and those of interested organisations outside the system. Provision is being made for science writers and other journalists and possibly, if space allows, for members of the public. This is intended as a serious scientific meeting, the output of which will include a message from the world scientific community to UNCSTD, as well as proposals relevant to the conference programme of action.

Besides various papers to be prepared by the UN Office of Science and Technology, which is providing the secretariat for this meeting, there will be important inputs from four symposia being held within the context of UNCSTD between now and next summer. These include that at Tallin, on Trends and Prospects with regard to the Development of Science and Technology (4-8 December 1978); Science and Technology for Development (Singapore, March 1979) organised by a group headed by ICSU; Technology for Development (Abidjan, Ivory Coast, April 1979); and Science and Technology in Development Planning (Mexico, May 1979).

Priority among subjects discussed at the present ACAST meeting went to those on which the committee's views had been requested by José da Costa, Secretary General of next year's UN Conference on Science and Technology



for Development (UNCSTD). Insofar as the conference itself is concerned, the most important of these was the discussion of "obstacles to the application of science and technology for development". Having as a basis a list of 31 general areas in which such obstacles might arise, ACAST pointed out that this list could be expanded to a hundred or more. It considered them by categories, each of which might be susceptible to the same type of solution at national, regional, or international level. They then fell into two broad groups: political, financial, economic and institutional obstacles to development; and those involving education and human resources, the availability and dissemination of information, and the psychological problems inherent in any society subject to the pressures inseparable from rapid development.

ACAST's suggestions stress the need for an integrated approach to specific problems that are seen as capable of reasonably early solution. Thus, to give a few examples: to improve the communication gap in a given country between its scientific and technological community, decision-makers and users, a well-mandated national advisory council might be set up, that would monitor trends and developments and interpret them to the leadership, as well as interpret political goals and objectives to the scientific and technological community. At the regional level, programmes of common interest could be identified as part of regional economic planning policy, with a regional machinery to coordinate and evaluate their performance. At a practical level, regional servicing facilities might be established, to maintain and service the special equipment of all developing countries in the region; it could maintain a spare parts bank to avoid the harassment of delays in obtaining equipment spares. With regard to in-

formation at both regional and international levels, there should be a network of information systems not only to act as a data bank, but also to assist in collecting trends of development in technologies and to interpret their implications for developing countries.

ACAST concluded that "at the national, regional as well as international levels, the psychological obstacles can only be removed by redesigning and strengthening the education, training and information systems".

Recognising social science

Finally, ACAST briefly discussed its own future. Obviously, if something like the UN Centre for Concerted Action and Coordination on Science and Technology described above is set up, there would be a function for a small body similar to ACAST, and this is already foreseen in the suggested "committee of top-level experts". One point of which ACAST is now well aware is the need to include, in whatever body replaces it after UNCSTD and as a result of the restructuring exercise, representatives of the social sciences. The intention was never to include these when ACAST was set up in 1964, but their presence is at last seen as essential if a balanced view is to be obtained of what science and technology are doing, can and should be able to do, in the world as a whole. This much is now recognised. But whatever the decisions of the General Assembly, which has the final say in ACAST's future, it is certain that some such body must exist if the UN Secretary-General, essentially preoccupied with political affairs and the in-fighting of the UN system, is to be kept aware of what is happening in the science and technology on which the world increasingly and inevitably now depends. □

Racial resegregation measured

DESPITE decade-long efforts to integrate schools, increase individual welfare payments and revitalize central cities, the underlying conditions attacked by these programs remain almost unabated, according to three reports delivered at last week's meeting of the American Sociological Association in San Francisco.

A new study of school integration and the so-called "white flight" of middle class caucasian families to suburbs from the city centres reestablishes the seriousness of this problem, first treated in a controversial report by James Coleman in 1975. Coleman's treatment was severely criticized at the time for neglecting concurrent demographic changes and not distinguishing between forced and voluntary desegregation.

Now, David J. Armor of the Rand Corporation, Santa Monica, California, has analysed integration statistics by estimating what out-migration of whites would have taken place anyway, taking into account the different birth rates between whites and blacks, and comparing the results of court-ordered busing to voluntary methods of desegregation. He concludes that forced integration accelerates white flight by a factor of from two to four, but that voluntary integration may not accelerate it at all.

He compares specific cases by using a "desegregation index", defined as the average percentage of white students in schools attended by minority students. (If minority students were divided among all American schools completely randomly, each school would have a desegregation index of 70 to 80. The smaller the index, the less integration.)

Armor found that, with the beginning of court-ordered integration, the index for a particular school district usually jumps. In one year, the desegregation index of Pasadena, California, rose from 37 to 53. But over the next seven years, it steadily dropped back to 35, in 1977. By comparison, nearby San Diego started its voluntary busing plan in 1968 with an index of 43. The index rose gradually to 46 and has now slipped back to 44.

During roughly the same period, a variety of programs were introduced to help raise the average income of blacks and other minorities. A report by Robert B. Hill of the National Urban League Research Department shows that again many early gains have been eroded by the "benign neglect" policies of the Nixon and Ford Administrations and the two severe recessions of the early 1970s.

John Douglas

correspondence

Sunspots and flu: a correlation

SIR,—Seasonality exerts a profound but ill-understood influence on epidemic influenza. North of the tropic of Cancer and south of Capricorn epidemics usually break out in winter when the relevant portion of the globe of the Earth is most distant from the Sun. The purpose of this note is to call attention to a longer-term association of type A influenza with solar activity that has become apparent since human influenza virus was discovered in the epidemic of 1932–33, namely that the periods of world-dominance of successive major subtypes of influenza A virus have synchronised closely with the periodicity of sunspots (see Figure). The two sets of cycles are probably synchronised at least as far back as 1917.

Influenza pandemics have occurred in the twelve months after type A influenza virus has undergone an antigenic shift leading to a new major subtype. The new major variant with altered haemagglutinin (H) and neuraminidase (N) finds the population inadequately protected by influenza caused by its predecessor. The change has enabled it to bypass their immunity. The predecessor promptly disappears and the new major variant with its minor varieties dominates the world causing all the type A influenza for the next decade or so until itself displaced by the next antigenic shift. The major subtypes since 1933 have been identified serially according to H & N composition as H_2N_1 viruses isolated before 1947, H_1N_1 1947–1957, H_2N_2 1957–1968, H_3N_2 1968–1978. Each major subtype completely disappeared at the next antigenic shift except that H_1N_1 viruses reappeared in many parts of the world in 1977 and are still (28 July 1978) causing influenza concurrently with H_3N_2 viruses. The pandemic of 1918 is usually attributed to antigenic shift producing the major subtype HSw_1N_1 . A small outbreak caused by virus of this major subtype recurred in 1976.

Sunspots undergo cycles of activity varying from seven to seventeen years,

average eleven years. Activity increases to maximum more rapidly than it declines to minimum, and maxima sometimes stand upon a plateau of several years of approximately equal activity. The true solar sunspot cycle consists of two consecutive cycles of minimum to minimum activity (see Gibson, Edward G. *Rev. Geophys. Space Phys.* **10**, 395–462; 1972). Antigenic shifts of influenza A virus to H_1N_1 , H_2N_2 and H_3N_2 coincided with sunspot maxima of 1947, 1957 and 1968 respectively. The sunspot maximum of 1937 coincided with severe and widespread influenza, but no antigenic shift was recorded. The sunspot maximum of 1928 coincided with a pandemic which may have signalled the antigenic shift from HSw_1N_1 to H_0N_1 virus. The previous sunspot maximum of 1917 anticipated the pandemic of 1918–19. Fascinating as it is to attempt to investigate the association throughout the centuries of sunspot records, influenza records are probably too dubious to make the attempt worthwhile. The next sunspot maximum is however to be expected shortly, perhaps in 1979. The behaviour of type A influenza virus during the next few seasons will be watched with keen interest tinged with apprehension.

Yours faithfully,

R. E. HOPE-SIMPSON

Epidemiological Research Unit,
Cirencester, UK

Racism

SIR,—Professor Eysenck's clear dissociation from the National Front and other 'explicitly' racist organisations is to be welcomed, and I assume that he will be taking steps to ensure that the organisation and its associated journals cease using his name. In the meantime, although I am sure that when he denies that he has given an interview to *The Beacon* he does so in good faith, a further clarification seems required, as I have in my possession a xerox copy of an issue of

The Beacon containing an interview in the form of questions and answers between someone described as a *Beacon* reporter and someone *The Beacon* describes as Professor Eysenck. Could the granting of this interview, by one who gives so many, have slipped his mind?

However, the most serious problem with the answers to my letter (*Nature* **274**, 738; 1978) is that both Professors Eysenck and Jensen fall yet again into the typological trap of the confusion of social and biological categories. To speak of "racial mean differences" to is do the reverse of to "treat each person individually", which Professor Eysenck claims he wants; it is to categorise them as belonging to a group, which although socially defined (eg, Black, Jewish) Professors Eysenck and Jensen regard as having biological meaning. The resulting scientific errors which flow from their attempts to biometrise group characteristics are apparent to most biologists. As the National Front propaganda has shown, racist ideology feeds on this biologising thinking, and it needs firm public rebuttal.

Yours faithfully,

STEVEN ROSE

Open University,
Milton Keynes, UK

Origins of "ecology"

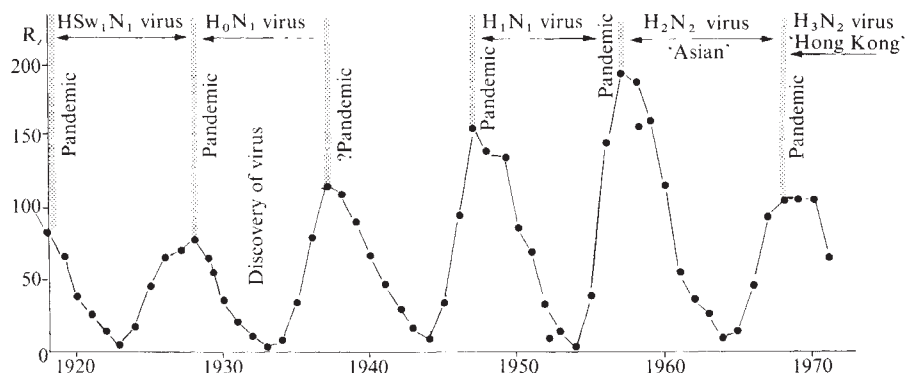
SIR,—In reviewing the book by D. Worster, *Nature's Economy*, Kenneth Mellanby (*Nature*, 6 April 1978) states that Haeckel "is believed to have coined the word [ecology] in 1873". I do not know if the word was used earlier, but it is certainly true that Ernst Haeckel in his volume, *Generelle Morphologie der Organismen*, Berlin, 1866, Vol. 2 ("Allgemeine Entwicklungsgeschichte der Organismen"), p 286, states: "Unter Oecologie verstehen wir die gesammte Wissenschaft von den Beziehungen des Organismus zur umgebenden Aussenwelt". He therefore gives the correct definition of ecology, as we understand it today. Haeckel goes on (p 287): "Dagegen hat sie die Beziehungen desselben zur Aussenwelt, die Stellung, welche jeder Organismus im Naturhaushalt, in der Oeconomie des Natur-Ganzen einnimmt, in hohem Grade vernachlässigt".

Haeckel's definition of ecology, integrated with the concept of ecology as the study of the "economy of nature", is repeated in Haeckel's popular collections of lectures, *Natürliche Schöpfungsgeschichte* (1868), and *Anthropogenie* (1874). Both books were translated into many languages, and had dozens of editions, containing increasing numbers of 'lectures'. Readers may like to refer to a short history of the concepts of 'ecology' and of the 'economy of nature' in my paper: "Ecologia e economia", *Giornale degli Economisti*, **32**, 435–455 (1973).

Yours faithfully,

GIORGIO NEBBIA

Università di Bari, Italy



Sunspot cycles and major variants of influenza A virus. Changes of major variant occurred abruptly with disappearance of predecessor variant. R_z : Zurich yearly means of daily Relative Sunspot Numbers

news and views

Mussel watching

from B. L. Bayne

BIVALVE molluscs, particularly mussels and oysters, are valuable indicators of pollution in coastal marine environments and estuaries. Among their qualifications for this role are their sessile mode of life as adults and a capacity for accumulating contaminants in their tissues to levels considerably higher than in seawater. The first attribute makes escape from various environmental stresses impossible. The second makes bivalves attractive to analytical chemists; whereas most pollutants may be present in seawater in concentrations between 10^{-15} and 10^{-12} g g⁻¹, concentrations in bivalve tissue may vary between 10^{-9} and 10^{-4} g g⁻¹. Mussels of the genus *Mytilus* fit this role of indicator organism particularly well because they provide convenient amounts of tissue for analysis and because they are common members of benthic (often intertidal) communities on hard substrata, with a wide and circumpolar distribution in boreal and temperate waters of both northern and southern hemispheres.

In 1975 Ed Goldberg, of the Scripps Institution of Oceanography, proposed a 'mussel watch' in which collections from many sites would be analysed regularly for their body-burdens of heavy metals, halogenated hydrocarbons, petroleum hydrocarbons and transuranic nuclides. The results would provide "a continuing revelation of how man's activities are altering oceanic composition" (*Mar. Poll. Bull.* 6, 111; 1975). In 1976 this idea bore fruit with a surveillance programme in the USA, under contract with the US Environmental Protection Agency. Samples of mussels or oysters are taken from 107 stations on the West, Gulf and East coasts of the US and analysed in five laboratories, each main class of contaminant being treated independently by two analytical groups. The results of the first year's work have now been published by Goldberg *et al.** The report

includes a discussion of sampling strategies, of some of the problems associated with seasonal and size variability, and of the economics involved in sampling and analysis, as well as providing detailed tables and discussions of the results.

In projects of this kind care is needed in designing the sampling procedure in order to ensure true compatibility between sites. Ideally, animals should be taken at a standard size, since the accumulation, of metals at least, is known to be size dependent (Boyden *J. mar. biol. Ass. U.K.* 57, 675; 1977). Sampling should be limited also to a standard tidal level on the shore and to a particular season of the year (Phillips *Environ. Pollut.* 13, 281; 1977). When the project covers a very wide geographical area, these may be difficult constraints to meet. Goldberg *et al.* sampled monthly at two stations and found no significant seasonal trend in concentrations of heavy metals or halogenated hydrocarbons, although a seasonal change in ^{239,240}Pu and ²⁴¹Am was interpreted as due to a change in the environment rather than a seasonality in the physiology of the animals. There have, however, been other reports of seasonal changes in the contamination of mussel tissue, a result to be expected knowing the large seasonal changes in metabolism that occur in bivalves associated with gametogenesis and the availability of food. In a widely based mussel watch it would be wisest to include independent checks for possible seasonal effects.

Another problem is posed by the treatment of the samples before analysis. Mussels feed on suspended particulate material (Goldberg *et al.* suggest that this is the main route for contamination by radionuclides) and, particularly in estuaries, their stomachs are likely to contain much inorganic material that would normally be passed out in the faeces. Consequently, a period of 12-48 h spent in clean seawater between sampling and analysis, in order to clear the gut by defaecation, is generally to be recommended.

But this is not practicable in a project on the scale of the US mussel watch, where samples were frozen on dry ice immediately following collection.

Another potential problem, that of calibration between laboratories, was specifically addressed in the US mussel watch, and the results are generally encouraging. Agreement between analytical laboratories was satisfactory for most of the metals and for the transuranic nuclides. Consistent differences occurred between laboratories in the analysis of the two principal degradation products of DDT, p,p'-DDE and p,p'-DDD. These differences are not viewed as alarming, particularly since all samples were sent for analysis to each laboratory chosen to analyse the particular contaminant. However, the need for careful intercalibration studies in future programmes is emphasised.

Having measured the concentration of each contaminant in all samples, a decision on whether the environment is 'polluted' must be made. It is on this point that some argument is to be expected. Goldberg *et al.* recommend that order of magnitude differences (or variations over time) should be considered significant when considering metals or halogenated hydrocarbons. On this basis particular pollution 'hot-spots' are recognised, for example the New York-New Haven area for metals and numerous sites of PCB pollution on the East coast. For PCB 1245, relatively unpolluted areas show tissue levels less than 50×10^{-9} g⁻¹ dry weight, whereas polluted areas have levels as high as 600×10^{-9} – 800×10^{-9} g g⁻¹. This criterion of an order of magnitude difference in order to discriminate between polluted and unpolluted sites is conservative, but rigorous enough in the context of the US mussel watch data; how generally applicable it may be remains to be seen. In setting such a criterion, however, the need for samples from a large number of sites is emphasised. As Gold-

B. L. Bayne is at the Institute for Marine Environmental Research, Plymouth.

*Goldberg, E. W., Bowen, U. T., Farrington, J. W., Harvey, G., Martin, J. H., Parker, P. L., Risebrough, R. W., Robertson, W., Schneider, E. & Gamble E. *The Mussel Watch, Environ. Conser.* 5, 1-25 (1978).

berg *et al.* point out, some locally high values for metals, in particular, may be due to natural estuarine run-off (see Bryan & Hummerstone *J. mar. biol. Ass. U.K.* **58**, 401; 1978) rather than industrial pollution. This is one aspect of the perennial problem in pollution monitoring, the setting of natural 'base-lines' for particular contaminants. Only by treating many samples from a large number of sites can a real appreciation be gained of true minimal concentrations of pollutants, and this must be particularly true in the coastal waters of industrialised states.

Using these and other criteria, the US mussel watch has identified certain areas of pollution; some of these conclusions came as no surprise, others remain more of a mystery. The study demonstrates the usefulness of the concept and argues strongly for an even wider geographical coverage. Indeed, Goldberg's original aim was for a global marine monitoring programme. The interest already shown by some countries (the proposed Mediterranean mussel watch, for example) suggests that an increase in geographical scope can be achieved.

This prospect poses a challenge for the biologist. Measuring the levels of

contaminants in the tissues of molluscs does provide information on levels of contamination of the water, but the question naturally follows: "What is the biological impact of these pollution loads?" To some extent, chemical studies of pollution require biological confirmation, for it is in the intensity of the effect on the biota that questions of pollution acquire environmental realism. Means of assessing the biological effects of pollutants are gradually being made available (*On the feasibility of effects monitoring, Co-operative Research Report No. 75*, ICES; 1978) and they vary from measures of biochemical response through indices for assessing the physiological condition of individuals to attempts to relate components of animals' responses to the possibilities of lasting ecological damage. Much of this research is focused on marine bivalve molluscs for the same reasons that render these animals attractive to analytical chemists. In promoting a wider application of the mussel watch concept, therefore, attention should be given to the incorporation of measures of biological impact. Goldberg *et al.* comment briefly on this point in their article, but it is a challenge to which the biologists need to respond. □

Autoimmunity revisited

from Noel R. Rose

It is now some 25 years since autoimmunity emerged as a plausible cause of a number of mysterious human diseases. But, despite the demonstration of autoantibodies in patients' sera or localised in affected tissues, there has been little understanding of how the autoimmune process gets started. The initiating event in human disease is certainly not deliberate injection of autologous antigen mixed with a suitable potentiating agent like complete Freund adjuvant, as it is in experimentally immunised animals. It now seems likely that one of the important prerequisites of autoimmune disorders in the human is genetic predisposition.

Early evidence that genetic abnormalities predispose to human autoimmune diseases stemmed mainly from two kinds of studies. Astute clinicians noticed that certain autoimmune diseases cluster with unexpected frequency in some families. When objective markers of autoimmune disturbance became available in the form of tests for autoantibodies, a statistically significant increase in auto-

immunity was documented even in clinically unaffected relatives of autoimmune patients. A recent line of evidence to support a genetic predisposition towards autoimmunity has come from investigations relating autoimmune diseases (as well as quite a number of non-immunological diseases) to genetically determined cell surface markers, especially HLA alleles. While rarely firm enough to be of practical diagnostic value, these statistical associations point to the existence of disease-susceptibility genes.

The World Health Organization Collaborating Center for Autoimmune Diseases, located at Wayne State University, sponsored a Workshop on Genetic Control of Autoimmune Disease in July 1978.* The purpose of the meeting was to see if the newly generated information derived from genetic investigations might open novel approaches to the diagnosis and treatment of human autoimmune disorders.

To begin, P. Terasaki (University of California, Los Angeles) and A. Svegaard (State University Hospital of Copenhagen) analysed the voluminous evidence associating autoimmune disorders with particular HLA alleles.

Large-scale population studies have clearly shown that a disproportionate number of individuals with particular HLA alleles develop certain diseases. Putative disease-susceptibility genes are spread all along the HLA complex, but the closest associations are with alleles at the D locus. For example, Dw2 is increased in multiple sclerosis, and Dw3 in a number of different autoimmune diseases, including thyrotoxicosis (Graves' disease), spontaneous adrenal insufficiency (Addison's disease), and pernicious anaemia. These same diseases show a significant but somewhat reduced association with one of the antigens in the B series, B8. This finding is most easily explained by assuming that B8 and Dw3 do not sort randomly; that is, are in linkage disequilibrium. As I. Roitt (Middlesex Hospital, London) pointed out, rheumatoid arthritis is unusual in that it associates with Dw-4 and shows no significant affinity for antigens of the A, B, or C series. Although statistically significant, these HLA associations are still distant enough to indicate that other genes and non-genetic factors greatly influence the development of autoimmunity. For example, genetic disorders in the particular target organ or sex-associated factors (these diseases are almost all more common in females than in males) are probably involved.

Sometimes HLA typing helps to distinguish different varieties of what was thought to be the same disease. Two different forms of diabetes mellitus can now be distinguished by HLA typing. The so-called juvenile-onset or insulin-dependent form of diabetes is associated in Dw-3, while individuals without this genetic marker are more likely to develop a late onset form of disease that can be controlled by diet or oral hypoglycemic agents, not requiring insulin. As J. Irvine (University of Edinburgh) emphasised, distinction between the two forms of diabetes should now be made, not on the basis of age of onset, but on HLA type and immunological response to pancreatic islet cells. HLA typing is also useful in prognosis; for example, those patients with Graves' disease who are HLA-B8 are less likely to undergo spontaneous remission than patients lacking this trait. I. Mackay (Hall Institute, Melbourne) distinguished two forms of chronic active hepatitis on the basis of immunological response. The autoimmune type of the disease shows an increase in HLA-A1, B8 and Dw-3. No such HLA association is found in chronic active hepatitis patients who are positive for Australia antigen.

*The meeting was organised by N. Rose, P. Bigazzi and N. Warner. The full proceedings are being published by Elsevier-North Holland.

Noel R. Rose is Professor and Chairman in the Department of Immunology and Medicine, Wayne State University.

The most likely role of HLA in determining susceptibility to disease is through immune response genes that determine recognition of a particular antigenic determinant. Evidence for this concept is based mostly on the close resemblance of HLA to mouse H-2, which is known to control immune responses.

Among the models available for the study of spontaneously appearing autoimmunity in animals, the New Zealand (NZ) strains of mice have been by far the most profitable. The NZB strain spontaneously develops haemolytic anaemia, and the hybrid obtained by crossing NZB with the apparently normal NZW strain results in an autoimmune disease closely resembling human systemic lupus erythematosus (SLE). Extensive efforts have been made to sort out the several genetic factors controlling these autoimmune disorders, but a clear definition has not yet been obtained. To complicate matters, NZ mice contain abundant type C virus particles. Viral genes code for glycoprotein which closely resembles a mouse glycoprotein, and the possibility was raised that antibody originally induced to viral proteins may cross react with the comparable mouse antigen. However, S. Datta (Tufts University) showed that viral genes segregate independently of 'autoimmunity' genes, removing the possibility of a cause-and-effect relationship between viral infection and autoimmunity.

A more likely possibility to explain the spontaneous development of autoimmune disease in NZ mice is a fundamental disorder in immunological regulation. H. Cantor (Harvard Medical School) demonstrated an early deficiency in the subset of T lymphocytes responsible for immunological equilibrium. These cells, recognised by their antigenic surface markers, Ly 123⁺, produce feedback suppression of antibody production. N. Talal (University of California, San Francisco) further showed that the paucity of suppressor T cells in NZ mice can be at least partially remedied by thymic extracts. Another view of the fundamental NZ abnormality emerged from experiments of H. Morse and T. Chused (National Institutes of Health). B cells of NZB mice show spontaneous polyclonal activation even in the absence of T cells. These findings suggest that NZ mice suffer from an intrinsic lesion in their B cells. There may be a more basic stem cell defect affecting both regulatory T cells and B cells.

In contrast to the multiple autoimmunities present in NZ mice, a single autoimmune disorder can be demonstrated in the OS strain of chickens. These animals spontaneously develop a classical form of chronic thyroiditis. L. Bacon (Wayne State University)

DNA structure and transcription

from Andrew Travers

THE mechanisms controlling transcription during the development of different bacteriophages are extremely diverse. Yet another solution has emerged from the studies of Rothman-Denes and her colleagues on coliphage N4. N4, in common with *Bacillus subtilis* phage PBS2, but unlike all other commonly studied phages, does not apparently require the host RNA polymerase for any viral transcription. Instead the virus contains a virus-coded transcriptase of novel structure. This enzyme consists of a single protein chain about 4,000 amino acids long and is thus one of the largest polypeptides so far identified.

How does this enzyme function? Its equally novel template requirements are now reported in the latest paper from Chicago (Falco *et al.* *Proc. natn. Acad. Sci. U.S.A.* **75**, 3220; 1978). *In vitro* N4 RNA polymerase strongly prefers N4 DNA, which it copies asymmetrically, producing transcripts corresponding to those RNA molecules synthesised *in vivo* immediately after phage infection. However, in contrast to other bacteriophages, the enzyme has the virtually absolute requirement that this template be denatured. Another pointer to the unusual nature of the specificity of the enzyme is the observation that *in vivo* N4 RNA synthesis is specifically inhibited by coumermycin, a drug whose target is DNA gyrase, the enzyme responsible for inserting negative superhelical turns into closed circular duplex DNA. These results suggest that DNA gyrase must modify the structure of N4 DNA before it can be transcribed by the virion RNA polymerase. Consequently, the authors propose that superhelical DNA may be the required natural template for the enzyme.

Assuming this hypothesis to be correct, we have to ask how the introduction of negative twists into presumably closed circular N4 DNA

so dramatically promotes transcription. The best current analogy comes from studies on *Escherichia coli* RNA polymerase. Before initiating transcription this enzyme recognises a promoter site extending over a region of at least 40–50 base pairs, and subsequently effects a change in DNA conformation at the binding site, a process termed promoter opening. It is well established that increasing the superhelical density of phage DNA templates substantially enhances the rate of transcription initiation by the bacterial polymerase. This enhancement could result from a direct facilitation of enzyme-mediated promoter opening. Alternatively the introduction of superhelical turns could alter the parameters which the enzyme recognises in the DNA template sufficiently to change the initial affinity of the polymerase for a promoter site.

At present the available experimental evidence does not distinguish between these possibilities. However, Levitt (*Proc. natn. Acad. Sci. U.S.A.* **75**, 640; 1978) has recently calculated that supercoiling can, in theory, alter the number of base pairs per turn of the double helix by as much as 5%. If this result is applicable to the real world, it is difficult to escape the implication that the spatial arrangement of the DNA sequence in a supercoiled molecule differs substantially from that in a relaxed one. Consequently unless one invokes the additional *ad hoc* assumption that RNA polymerase possesses a similar molecular flexibility it seems highly plausible that the detail of the initial contacts of the enzyme with a given promoter site will strongly depend on the extent of superhelicity of the template. However, the capacity of the bacterial polymerase for differential promoter recognition can be extensively modulated by factors other than subtle variations in DNA structure. Under these circumstances the N4 virion RNA polymerase promises to provide an attractive simpler system to test for the effects of supercoiling on promoter recognition and opening.

Andrew Travers is at the MRC Laboratory for Molecular Biology, Cambridge.

found that the trait is polygenic but is significantly linked to the B locus (the chicken's MHC) in certain families. In other families the B locus linkage may be obscured due to over-riding effects of other genes. Other genetic defects have been found in the OS thymus and in the thyroid gland itself.

On the basis of the many studies of spontaneously developing autoimmune disease in animals, three types of genetic eccentricity can be recognised. One is an increase in immunological responsiveness, possibly due to the presence of immune response genes within the MHC. Such a

gene is probably present in the OS chickens. A second lesion appears in the thymus and in the regulatory cells developed in the thymus. A third genetic defect probably occurs at the level of the target organ and determines where the particular pathological damage may be found.

Among the models of induced autoimmunity in animals, the most clearly defined is experimental allergic encephalomyelitis (EAE). D. Gasser (University of Pennsylvania) found that in the rat the development of disease is associated with the MHC, but other non-MHC related genetic controls can also be recognised. In mice the situation is even less clear-cut, because natural suppressor cells have a major, and possibly over-riding, influence. In some strains it is possible to detect genetic control by reducing the effect of T suppressor cells with cyclophosphamide treatment (R. Arnon, Weizmann Institute).

Another good model for studying genetic control of induced autoimmune responses is experimental thyroiditis. In mice, where many genetically defined inbred strains are available, Y. Kong (Wayne State University) was able to delineate at least two genes that regulated the response to mouse thyroglobulin. One, located near the K end of the H-2 complex, appeared to be a typical immune response gene. A second-order gene near the D end of the complex modified the immune response so that the pathological damage to the thyroid was heightened or diminished.

H. Wekerle (Max Planck Institute, Freiburg) has developed a method of autosensitising T lymphocytes *in vitro*. His experiments strongly suggest that self-responsive precursor T cells are normally present in the body but held in check by homeostatic immunological mechanisms. Further evidence that self-responsive lymphocytes are normally present to testicular antigens emerged from experiments described by P. Bigazzi (University of Connecticut), who found that vasectomy of rats and mice engenders production of sperm-specific antibody, a response also reported in vasectomised men.

Until recently, most immunologists believed that the lack of response to self-antigens came about because lymphocyte clones that carried receptors to self-antigens were eliminated at birth. The investigations described during the Cranbrook meeting contradicted this view. The data showed that both T cells and B cells capable of reacting with many self-antigens are present and waiting to be triggered. The prevention of autoimmune responses, therefore, requires continuous and active suppression.

Two regulatory mechanisms were

discussed in some depth. The first is the generation of antibodies to idiotypes, as described by H. Wigzell (University of Uppsala). Idiotypic determinants characterise antibody molecules and immunocompetent cells of a particular antigenic specificity. Idiotypic antibodies are strong candidates for physiological regulators of immune response, as they can control production of antibodies with particular specificities by stimulating or inhibiting idiotype-bearing immunocompetent cells.

Immunological regulation also depends on the balance of suppressive and stimulatory subsets of T cells. The balance depends on the properties of the antigen and the method of administration. R. Swanborg (Wayne State University) found that certain determinants of the EAE antigen favour suppressor cell production, specially if given with incomplete rather than complete Freund's adjuvant. E. Engleman (Stanford University) reported that he could generate *in vitro* a factor from human T cells that suppressed allogeneic lymphocyte interactions. The suppression is genetically

restricted by the D locus of the reacting cells, re-emphasising the crucial role of the MHC in immunological regulation.

To many participants, the meeting at Cranbrook foreshadowed a new stage in the thinking about human autoimmune disease, with a shift from descriptive to interventionist approaches. Until now, genetics has contributed mainly to understanding the natural history of autoimmune disorders, offering improvement in diagnosis and classification. Now it seems that the tools may be at hand to arrest the pathological process. One most promising lead is the deliberate generation of suppressor T cells *in vitro*, possibly followed by hybridisation with established cell lines and production of suppressor factors. Another possibility is to induce idiotypic antibodies with restricted specificity for self-reactive immunocompetent cells. The coming years should provide several opportunities for designing specific immunotherapeutic manoeuvres rather than relying on nonspecific immunosuppression for the treatment of autoimmune diseases. □

The decline and fall of protein chemistry?

from Alan D. B. Malcolm

FOR more than 20 years the techniques of protein chemistry, including sequencing have played an enormous role in the development of biochemistry. It seems likely however that six papers in the *Journal of Biological Chemistry* (253, 5484; 1978) may be the zenith of this particular branch of science. These describe the complete sequence of β -galactosidase from *Escherichia coli*, which with a molecular weight of 116,349 is the largest protein so far sequenced. Its 1,021 amino acids gave rise to 72 chymotryptic peptides (*op. cit.* 5484), 24 cyanogen bromide peptides (*op. cit.* 5499) and 89 tryptic peptides (*J. biol. Chem.* 247, 5425; 1972) and the separation and ordering of these has taken A. Fowler, I. Zabin and colleagues at the University of California at Los Angeles eight years, and involved growing *E. coli* on [¹⁴C]lysine and [¹⁴C]arginine as well as [³H]methionine to increase the sensitivity of detection. The techniques of nucleic acid sequencing have developed so quickly that this is now much easier than protein sequencing—a fact which is acknowledged at the end of the last paper (*J. biol. Chem.* 253, 5521; 1978) where the authors state that the

sequence of the first 145 residues agrees with that predicted from the DNA sequence determined in W. Gilbert's laboratory at Harvard. It cannot be long before the rest of the DNA sequence is used to confirm or correct the protein sequence.

In this, the β -galactosidase sequencers have a problem similar to that of Ovchinnikov's group in Moscow who set out several years ago to sequence the subunits of RNA polymerase. They published the sequence of the α -subunit (molecular weight 36,512) last year (*FEBS Lett.* 76, 108; 1977) but before they could finish the two large subunits (β and β' , with molecular weights of 155,000 and 165,000) comes the news from Nomura's laboratory in Madison that part of the DNA is being sequenced thus making the protein sequence almost (but not quite) redundant. Nomura is using transducing phage λ *drif*¹⁸ which contains *rpoB* and *rpoC*—the adjacent genes which code for β and β' . Identification of precise start points for the protein on the DNA is made a lot easier by the fact that the N terminal tetrapeptide sequence has been known for some time (Fujiki & Zurek *FEBS Lett.* 55, 242; 1975) and it seems likely that the protein chemist will still have to provide such short sequences in the future.

Alan D. B. Malcolm is in the Department of Biochemistry, St Mary's Hospital Medical School, London.

An interesting illustration of the changeover from protein to nucleic acid sequencing may be seen in the analysis of the phage λ repressor (Sauer & Anderegg *Biochemistry* **17**, 1092; 1978) where the authors have used a combination of the classical sequential Edman degradation, the more recent gas chromatography-mass spectrometry peptide sequencing and also the partial sequence of the *cI* gene (which codes for the repressor) obtained by the Maxam-Gilbert technique. The entire sequence of 236 residues was determined in the usual way and 179 of these were confirmed by GC-MS. The DNA sequence was then used both as further confirmation and also to resolve a disagreement with previous authors (Beyreuther & Gronenborn *Molec. gen. Genet.* **147**, 115; 1976) over the amino acids present at positions 14, 40 and 42. Like the *lac* repressor, the λ repressor binds to DNA through its N terminus and this region is highly basic with clusters of lysine and arginine at 3-5, 16, 17, 19 and 24-26.

Molecular analysis of the *lac* operon and its control regions is yet closer to completion with the recent publication of four papers (*Nature* **274**, 762; 1978) which include sequences for the promoter of the repressor gene (Calos *Nature op. cit.* 762) and the sequence of the repressor gene itself (Farabaugh *Nature op. cit.* 765). In both these papers the problem of obtaining enough DNA has been overcome by cloning restriction fragments shown to contain the relevant sequences in the plasmid vector pMB9, and again a transducing phage, λ h80dlac, which carries the *lac* operon, was useful as the original source of the DNA. The sequence of the repressor gene suggested that two peptides had been overlooked in the original sequence of the repressor (Beyreuther *et al. Proc. natn. Acad. Sci. U.S.A.* **70**, 3576; 1973), and also suggests that Gln should be Glu at positions 164 and 215. Beyreuther has checked the protein sequence (*Nature op. cit.* 767) and has found the missing residues—two are from a CNBr peptide at positions 231 and 232 and the other eleven at positions 148-158 are part of a tryptic peptide. Thus not only is DNA sequencing faster, but it is also less likely to lose fragments as a result of their small size or insolubility.

Of course it is still easier to purify a particular protein than to purify and characterise a particular segment of DNA but the latter is becoming easier all the time. In *Cell* (**14**, 673; 1978), Montgomery *et al.* report the isolation of the yeast cytochrome *c* gene. Several *EcoRI* restriction fragments of yeast DNA were found to hybridise to a synthetic oligodeoxynucleotide complementary to a region near the NH₂ terminus of the protein. A comparison

with the fragments obtained from a structural gene mutant where a G $\rightarrow$ T mutation abolishes an *EcoRI* site enabled the fragment containing the gene to be identified. The sequence of the first 91 nucleotides agrees exactly with the protein sequence. It will obviously not be long before phylogenetic trees based on nucleic acid sequences totally displace the less meaningful ones based on proteins.

Even the methods such as chemical modification developed to identify the residues vital for a protein's function now have a powerful rival in the technique of site-directed mutagenesis developed in Weissmann's laboratory in Zurich. This involves using a restriction enzyme in the presence of ethidium bromide to introduce a cut in only one strand of the DNA and then using the fact that DNA polymerase I will hydrolyse from the 5' end and re-synthesise at the 3' end of the break. By allowing this 'nick translation' to occur in the absence of some of the deoxynucleotide triphosphates and in the presence of a nucleotide analogue such as hydroxy dCTP (HOdC), the analogue may be incorporated just once at a predetermined position. Since HOdC can base pair with either G or A subsequent generations will either

be wild type or have a mutation at this site and the properties of the mutant may now be studied. So far this has been used to convert the QB phage coat protein cistron initiation sequence AUGGCA to AUAGCA or AUGACA or AUAACA and to study the effect on ribosome binding (Taniguchi & Weissmann *J. molec. Biol.* **118**, 533; 1978; *News and Views* **272**, 397; 1978). Perhaps surprisingly a G $\rightarrow$ A mutation in the fourth position actually increases the efficiency of ribosome binding whereas a G $\rightarrow$ A transition at the third position totally eliminates binding. The double mutant binds weakly. It seems as though the ability of the mutant A at position four to hydrogen bond to a U next to the CAU anticodon on fMet-tRNA must be responsible. Weissmann has now applied this trick to the gene for rabbit β -globin (albeit in a plasmid rather than in the animal) (Muller *et al. J. molec. Biol.* **124**, 343; 1978). Such an approach will clearly have a tremendous impact on attempts to establish structure-function relationships for proteins. Custom designed mutants seem likely to make much of today's elegant protein chemistry seem very crude. $\square$

Astronomers licked by QSOs and active galactic nuclei

from John Faulkner and Martin Gaskell

A RECENT workshop on quasars and active galactic nuclei hosted by Lick Observatory provided not only an appropriate topic for discussion at this centre of optical and theoretical work on emission line objects, but also provided a forum for the exchange of views (and occasionally insight) derived from other wavelength regions. Important contributions were made towards the resolution of long-standing problems; in other cases recently discovered problems became even more acute.

The subject is bedevilled for the non-specialist by the terminology used to describe the vast subclasses of objects observed (QSOs, BSOs, OSQs, BL Lacs, OVV's, NLRGs, BLRGs, AO 0235+164 objects and so on). It was not always clear that many of the participants bore the numerous fine observational distinctions between these objects in mind in their discussions, or even whether they should have. There did seem to be a tacit assumption that all QSOs and active galactic nuclei

(AGNs) are indeed manifestations of varying degrees of the same phenomenon.

The similarities and differences between radio-selected QSOs and optically-selected QSOs (OSQs) were discussed by several participants. Techniques for finding OSQs are improving. Less than 10% of these are turning out to be 'radio-loud'; and of the latter only a very small fraction are extended sources, which prompted D. Weedman's remark that "it's just ironic that radioastronomers found QSOs". A. Savage (Royal Observatory, Edinburgh) emphasised the problems of personal judgment in the selection of objects from prism plates. For example, one person might preferentially find high red-shifted, another low red-shifted OSQs on examining the same plates. It is therefore not yet possible to compare directly the surface densities of radio QSOs and OSQs. It does seem, however, that the surface density of OSQs increases to fainter magnitudes without any cutoff, in contrast to radio QSOs which have a peak in their magnitude distribution. Both Savage and P. Osmer (CTIO) remarked that

John Faulkner and Martin Gaskell are at the Lick Observatory, University of California, Santa Cruz.

radio-loud QSOs are far more likely to be optically variable than are radio-quiet QSOs.

No doubts at all were raised over the cosmological interpretation of emission line redshifts. Indeed almost the last nail in the non-cosmological redshift's coffin was hammered in by the acclaimed work of A. Stockton (Hawaii), who gave strong evidence for an association of galaxies with bright QSOs. Furthermore he showed that the redshift distributions of the associated galaxies were essentially symmetric about the emission line redshifts—thus lending great weight to the formerly disputed notion that the QSO emission line redshift is a 'true' cosmological redshift.

An important recent development in the often frustrated application of QSOs to cosmology has been the discovery and employment of the 'Baldwin effect' (a correlation between the CIV line strength and continuum luminosity; see Baldwin *et al.* *Nature* **273**, 431; 1978; *News and Views* **273**, 428; 1978). At the workshop it became increasingly clear that the Baldwin effect is most strongly seen in flat-radio spectrum QSOs, for which it was in fact first established. Osmer, for example, has now found faint weak-lined OSQs so that 'violation' of the effect by the radio-quiet QSOs can no longer be entirely attributed to an optical selection effect. In addition Harding E. Smith (University of California, San Diego) has identified a new subset of steep radio spectrum QSOs possessing unusually steep optical spectra (and of intrinsic interest in themselves) which do not satisfy the Baldwin effect. Nevertheless, C. M. Gaskell (Lick Observatory) reported that the Baldwin effect does seem to be holding up well in the face of new data for the flat radio spectrum objects. However, the use of the relationship to derive a value for the deceleration parameter q_0 of well over 0.5 provoked perhaps the most lively controversy of the workshop. Some support for a q_0 of around 1 came from R. F. Green (Caltech) in his application of the Baldwin effect to satellite ultraviolet observations of two flat radio spectrum QSOs. This holds promise, as further data comes in from the International Ultraviolet Explorer (IUE), that a wider redshift range can be used and applied to cosmology. Naturally, for that application it will be important to demonstrate that evolutionary effects are either absent or can be correctly allowed for. In this respect the infrared observations of Puetter (University of California, San Diego) and the IUE observations of J. B. Oke (Caltech) and Green were important for showing the similarities between high and low redshift QSOs when regions of the same rest wave-

length are compared.

All these observations accentuated recent problems in understanding the relative intensities of the hydrogen emission lines in both QSOs and Seyfert galaxies. Problems which had arisen earlier on a comparison of lines in composite spectra (which might therefore be suspect if QSOs of differing redshifts were dissimilar) were brought into stark focus now as it became clear that the Ly α to H β line ratios differed from those predicted by recombination theory by a factor of ten, and that this could not be explained by simple external reddening alone. Despite the endeavours of H. Netzer (Texas) and R. London (JILA, Colorado), no single mechanism seemed able to produce the desired suppression of Ly α or enhancement of the Balmer lines.

The nature of QSO absorption lines (that is, whether they are due to intrinsic or intervening matter) remains unresolved. There are still a large number of unidentified lines, differing from QSO to QSO. A. E. Wright (A.A.O.) made the (serious) suggestion that progress might be achieved by assuming that all absorption lines blueward of Ly α emission which had formerly been assumed to be all Lyman lines, should instead be assumed not to be Lyman lines at all. A. Wolfe (Pittsburgh) reported the detection of a third 21-cm absorption line QSO.

G. K. Miley (Leiden) in collaboration with J. S. Miller (Lick), produced the first significant correlations between the optical spectra and radio morphology of QSOs. Extended sources have broader and more complex H β profiles. A direct outcome of the workshop was a collaboration between Miley and R. Angel (Arizona) which showed a striking correlation between the position angles of the optical polarisation and the large scale radio structure of the double sources.

No startlingly new theoretical ideas were produced, although many old scenarios have been pushed much further. In particular the detailed evolution of the dense nuclei of galaxies has been pursued by M. C. Begelman (Cambridge) and S. I. Blinnikov (Moscow). It seemed that all routes led to black holes.

The organisers had assigned M. J. Rees (Cambridge) the heroic task of chairing the session on complete models; a job which proved even more challenging when none was forthcoming. The model of R. D. Blandford (Caltech) and Rees, which tried to explain BL Lacertae objects as ordinary QSOs viewed with their highly relativistic jets pointing at us, met with firm opposition from the observers on whom these jets were supposedly aligned. □



A hundred years ago

The Sea-Serpent Explained

On Monday, August 5, a number of geologists crossed in the Folkestone boat to Boulogne, to study the interesting formations of that neighbourhood, and, when about three or four miles from the French coast, one of these gentlemen suddenly exclaimed, "Look at that extraordinary object passing across the bow of the steamer, about a mile or a mile and a-half in advance of us!" On turning in this direction there was seen an immense serpent, apparently about a furlong in length, rushing furiously along at the rate of fifteen or twenty miles an hour; it was blackish in front and paler behind; its elongated body was fairly on the surface of the water, and it progressed with an undulating or quivering motion: *mirum erat spectaculum sane*.

Of course many suppositions were immediately started to account for this extraordinary phenomenon, but they quickly changed and settled into the fixed idea that the object before them could be nothing less than the great sea-serpent himself; for,—

"Prone on the flood, extended long and large,
Lay floating, many a rood, in bulk as huge
As whom the fables name of monstrous size,
Leviathan; which, God of all his works
Created hugest, that swim the ocean stream."

The writer fortunately had with him one of Baker's best opera-glasses, and, after a few moments' use of this little instrument, the wonder was satisfactorily resolved. The first half of the monster was dark and glittering and the remainder of fainter hue, gradually fading towards the tail. The glass did not determine the matter until the extreme end was reached, and then it was seen to consist of a mass of birds in rapid motion; those that were strong on the wing were able to keep well up with the leaders, and so make the head appear thicker and darker by their numbers, whilst those that had not such power of flight were compelled to settle into places nearer and nearer the tail. Doubtless these birds were shags (*Pelicanus cristatus*) returning to their homes for the night from the distant waters in which they had been fishing, during the day; perchance it may be wrong to assert positively as to the variety of bird, but inasmuch as the writer has often seen shags on the Cornish coast in smaller numbers returning in single or double file to their roosting places, and since it is stated in works of natural history that they have been noticed occasionally flying in this peculiar manner to the number of a thousand or more, it does not appear an unwarranted liberty in supposing that they really were *Pelicanus cristatus*.

From *Nature* **18**, 5 Sept., 489; 1878.

review article

Prospects for the use of amorphous semiconductors in solar energy conversion

J. I. B. Wilson & D. Weaire*

There is increasing interest in amorphous silicon as a low cost material for photovoltaic solar cells. Its possible uses extend to other methods of solar energy collection such as photothermal and photogalvanic conversion.

THE mutually stimulating but unpredictable interactions of pure and applied science are well exemplified by the history of amorphous semiconductors. These materials emerged from obscurity in the late 1960s, when new electronic switching devices, based on amorphous chalcogenides, excited widespread interest¹. Of course, this was not the first application of amorphous solids. They already had many uses, both mundane (for example, common glasses) and sophisticated (for example, amorphous Se, in xerography). Nevertheless, the inspiration for much of the concentrated academic activity in this area during the 1970s lay in the prospect of a new sub-field of electronics ('Övronics') where the established picture of a crystalline semiconductor might not be at all relevant. Important new concepts have emerged, such as that of Anderson localisation², but the theory of amorphous semiconductors remains a difficult subject, rarely united happily with experiment³.

Small wonder then, that many have preferred not to jump in at the deep end by exploring the switching effect in the multi-component glasses. There have been obvious attractions in the study of the elemental amorphous semiconductors, particularly Si and Ge. It was, therefore, chiefly in the context of these comparatively simple systems that the debate between the rival microcrystalline and random network models of microscopical structure was conducted⁴. The consensus now favours the latter as the better conceptual basis for the description of amorphous Si and Ge.

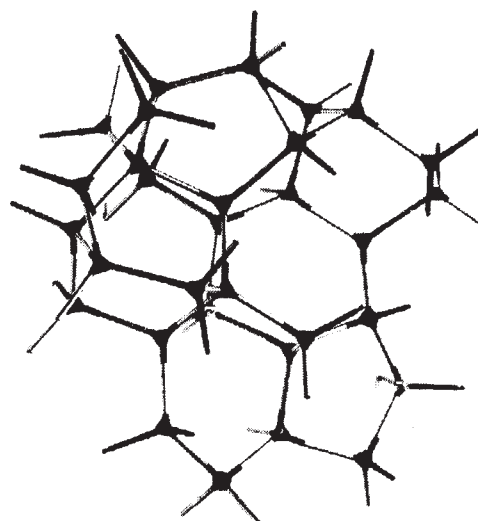
There have been no important applications of amorphous Si and Ge (apart perhaps from optical coatings, where a wide variety of materials find a use). However, it has recently been shown that amorphous Si, when suitably prepared, can be used to make conventional semiconductor devices⁵. This was first demonstrated using the r.f. glow discharge decomposition of silane to deposit the amorphous film and varying the doping by the admixture of gases containing p-type and n-type dopant atoms⁵. It was then realised that the incorporation of hydrogen which is incidental in this process is essential for its success. Various other methods (such as sputtering⁶), suitably adapted to incorporate a small proportion of hydrogen, are under investigation. It remains to be seen whether the glow discharge process will be preferred when these alternatives have been fully explored.

The role of hydrogen is to reduce the density of electronic states in the gap region (typically from $10^{20} \text{ cm}^{-3} \text{ eV}^{-1}$ to $10^{18} \text{ cm}^{-3} \text{ eV}^{-1}$). These states are presumably associated with 'dangling bonds' in hydrogen-free samples, to which the hydrogen atoms are bonded when present. Figures 1 and 2 give an impression of the general nature of this material—a random network in which some of the Si atoms have one or more H atoms bonded to them.

In this article, we review some potential applications of amorphous Si in the field of solar energy conversion. These are not confined to the junction devices mentioned above, that is, solar cells, but these certainly constitute the most promising prospect.

How can such an intrinsically messy material have advantages over crystalline Si, on which so much of our device technology is already based? The answer lies in the anticipation of a comparatively low cost for the industrial production of amorphous Si in thin films of large area. In this regard, solar energy research runs

Fig. 1 Fragment of a random network model of amorphous silicon, in which tetrahedrally coordinated silicon atoms form an infinite non-periodic structure.



*Department of Physics, Heriot-Watt University, Edinburgh, UK.

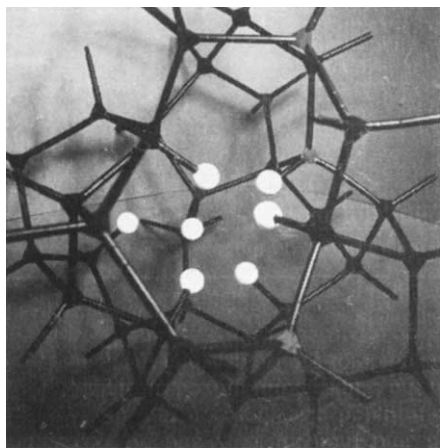


Fig. 2 Fragment of a random network model of hydrogenated amorphous silicon, with hydrogen atoms bonded to some silicon atoms.

counter to the trend of modern electronic device engineering in which 'small is beautiful'. The question then is whether acceptable efficiency and reliability can be obtained with an amorphous cell. This is still not clear but sceptics should be reminded that, had the single crystal window been invented first, they might be equally pessimistic about that familiar use of glass.

Photovoltaic conversion of solar energy

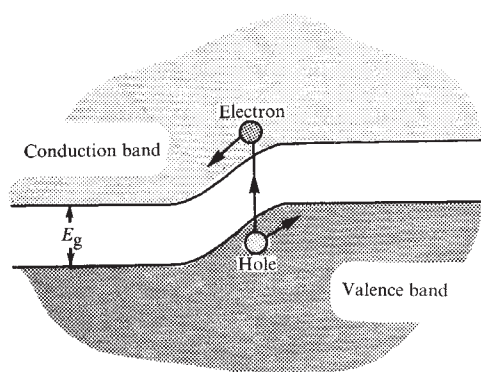
Photovoltaic cells, which convert light into electricity by a purely electronic process (that is, without chemical change) have been used for some years to power satellites. Indeed most of the development of high efficiency Si cells in the 1950s and 1960s derived from the needs of the space industry. They have also provided power in terrestrial sites where their convenience and reliability outweighs their high capital cost. Such applications will remain very limited until a much cheaper cell is developed. New research programmes in the US⁷ and Europe⁸ are aimed at this cost reduction.

All photovoltaic cells exploit the fact that a semiconductor will absorb radiation of sufficiently high frequency to excite an electron across the band gap, of magnitude E_g , and that electrons excited in this way return to the valence band ('recombine') only after comparatively long times (10–100 μ s).

In a p–n junction the energy levels associated with the valence and conduction bands vary in the manner idealised in Fig. 3, dropping by an amount E_g across the junction. Photogenerated electrons will move to the left (while the 'holes' left behind in the valence band move the other way).

If the junction is isolated (open circuit condition), the resulting charge imbalance will set up a potential difference V_{oc}

Fig. 3 Electronic energy levels at an idealised p–n junction. An electron–hole pair created by absorption of radiation near the junction is separated as shown (photovoltaic effect).



opposing the charge transfer. The system can only come into equilibrium when this exactly balances the change in electron energy levels at the junction, that is $eV_{oc} = E_g$ in our simplified picture.

This is the essence of the 'photovoltaic effect'. If the junction is incorporated in a circuit, a current I will flow and V will be correspondingly reduced. The power output is then simply IV and this is divided (for the optimum load) by the solar energy flux to give the maximum efficiency of the cell.

A narrow gap semiconductor absorbs most of the solar spectrum but wastes much of it as electrons are excited well above the gap and their surplus energy is merely converted into heat. On the other hand, a wide gap semiconductor fails to absorb at the infrared end of the spectrum. Thus the continuous frequency distribution of solar radiation (Fig. 4) enforces a compromise, favouring the choice of a semiconductor of band gap in the region of 1.5 eV. Even for this, the power efficiency is reduced to <50% by the waste at the low and high ends of the spectrum. Realistic models of junction behaviour¹⁰ reduce the theoretical efficiency to ~25% and practical considerations (grid contacts, and so on) reduce this figure still further. Most commercial Si cells achieve ~10–15%. The best laboratory result is 23% for GaAs cells in concentrated sunlight.

Schottky barrier solar cells are based on a metal–semiconductor junction (sometimes modified by the inclusion of a thin insulating layer at the junction). The essential structure of both types of cell is shown in Fig. 5.

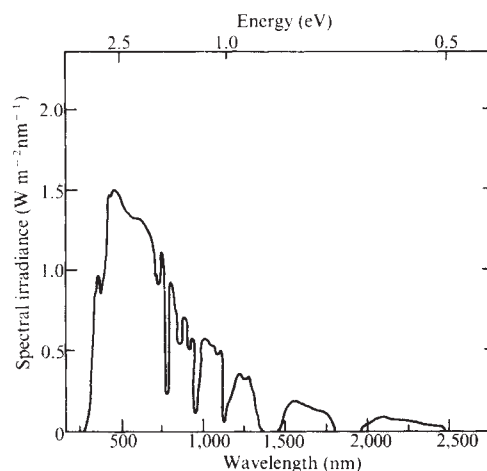


Fig. 4 Spectrum of incident solar radiation at the Earth's surface, for normal incidence (AM1).

Commercially available cells at present use semiconductor wafers sliced from expensive single crystal boules. Two routes are being taken to reduce the cost.

First, the addition of lenses or mirrors to an expensive cell may produce a cheaper module by concentrating sunlight upon the cell. This approach is most appropriate when a large proportion of insolation is direct. It is of particular value in association with GaAs cells, which are efficient solar energy converters at higher operating temperatures than silicon.

Second, silicon for solar cells does not require such perfection and stringent purity control as for most other semiconductor devices and so polycrystalline boules or extruded ribbons may be of adequate quality. Taken to its logical extreme, this approach leads to the consideration of polycrystalline or amorphous thin films.

On the whole, polycrystalline thin-film solar cells, using a variety of semiconductors, have been disappointing. Often only fine grained polycrystalline films can be grown with unsatisfactory electrical behaviour due to grain boundaries. In other cases, it has been difficult to grow uniform defect-free films more than a few mm^2 in area, and sometimes the cells have shown excessive degradation after a short period of operation.

Amorphous silicon avoids some of these problems and even has certain marginal advantages over crystalline silicon. For instance, it has a bandgap 1.5–1.7 eV, closer to the optimum than that of crystalline silicon (1.1 eV), and furthermore absorbs radiation more strongly just above the gap. Radiation hardness is sometimes mentioned (particularly in the context of satellite power stations). However, while it may be true that "one of the unique properties of amorphous semiconductors is their low sensitivity to high energy radiation..."¹¹ there is relatively little in the published literature relating to this topic.

Current laboratory work is concentrated on the achievement of acceptable efficiencies. 'Acceptable' is generally taken to mean at least 8% but depends strongly on the application and its location.

Within a short space of time various p-n and Schottky cells based on amorphous Si have been reported with efficiencies of the order of 5%, as summarised in Table 1. Theoretical estimates of the limiting efficiency of such cells have ranged from 8 to 15%, and it seems reasonable to expect that the lower figure can be attained in the near future. The factor which is most significant in reducing the efficiency in comparison with single crystal cells is the short diffusion length of the charge carriers.

Table 1 Performance of amorphous silicon solar cells, under AM1 or similar conditions

Cell structure	Open circuit voltage (mV)	Short circuit current density (mA cm ⁻²)	Efficiency (%)	Ref.
p-i-n	575	10.5	2.4	12
Pt-n	800	12	5.5	13
Ni-TiO _x -n	680	13	4.8	14

Only electrons and holes excited within about 1 μm of the junction survive to contribute to the photogenerated current. In a crystalline semiconductor the active layer may be as much as 0.5 mm thick. It remains to be seen whether this drawback is unavoidably inherent in the amorphous structure of the material or can be mitigated by refined preparative methods. However, little has been done (or, at least, published) on the optimisation of the doping profile close to the junction, which may yield some improvement.

It should be stressed that the results quoted above are for rather small area cells ($\approx 1 \text{ mm}^2$). It is a far cry from a laboratory cell of a few mm^2 to the orbiting solar power satellite suggested by Glaser¹⁵, with solar cells totalling 50 km^2 .

Photogalvanic and photochemical cells

Figure 6 shows the essential features of a photogalvanic cell. Again electrons are excited across the gap of a semiconductor at one electrode. Unfortunately most semiconductors with a suitable band gap are chemically unstable in electrolytes. TiO₂, a chemically stable compound which has featured in many studies, has a rather wide gap ($\approx 3 \text{ eV}$). Photochemical cells, in which TiO₂ has also been used, produce chemical fuels such as hydrogen from water. Promising results have recently been obtained with CdSe (ref. 16) ($E_g \approx 1.7 \text{ eV}$).

Amorphous semiconductors may offer new possibilities for such cells. Favourable factors are their ability to form strongly adherent thin films on a variety of substrates and low cost, which in this case might make regeneration economically feasible.

Photothermal conversion

Flat-plate solar collectors for heating water are installed in thousands around the world. These operate by the 'greenhouse effect', which is generally taken to mean that the selective transmittance of glass is the dominant feature. Most of the solar

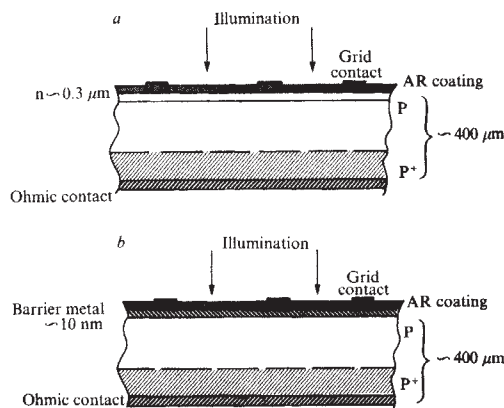


Fig. 5 Schematic structure of: *a*, conventional n-on-p silicon solar cell; *b*, metal-semiconductor (Schottky) junction cell. The dimensions are typical of crystalline solar cells.

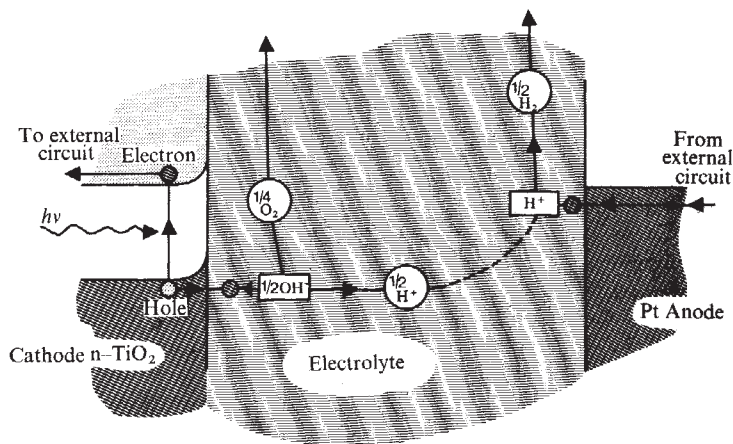
radiation passes through the glass but outgoing thermal infrared radiation, emitted by hot surfaces within the glass enclosure, is blocked. Consequently the temperature reached by an absorber plate is greater when a glass cover is in place. In practice the glass cover is equally important in reducing energy loss by convection, conduction, and evaporation, unless the temperature within is very high.

The cut-off point for transmission through glass lies around 3 μm , so some solar radiation is excluded from the enclosure, but a longer wavelength cut-off would mean that more energy would be lost by radiation. A compromise is to make the cut-off as sharp as possible, at about 2 μm . Although thin-film coatings can be applied to the glass to bring this about, there has been more interest in selective coatings for the absorber plate, for which the potential gain in collected energy is greater. A selective absorber will absorb visible radiation efficiently, but will not absorb infrared radiation. This means that equally it will emit visible radiation but not infrared.

Practical selective surfaces are generally semiconducting compounds, although their preparation may mean that their composition is obscure. Proprietary coatings include various Ni/Cr oxides/sulphides, which can have solar absorptances of up to 0.98 and infrared emittances of less than 0.2, even at the elevated operating temperatures.

Single-layer films such as these utilise the high infrared reflectance of a metal substrate (and consequently low infrared emittance) whilst providing an efficient absorber of visible radiation. It is only with multi-layer coatings that a sharp cut-off can be achieved, but this increases the complexity and cost of the

Fig. 6 Photogalvanic cell. An electron is removed from the electrolyte at the photo-cathode and one is injected into the electrolyte at the anode. Oxygen and hydrogen are evolved in this cell, by the simplified redox reactions shown.



collector. Semiconductors with a suitable bandgap for this application tend to have high refractive indices and are, therefore, a bad match to the low refractive index of air unless an anti-reflection coating is added. Sometimes it is possible to reduce the refractive index of a film by preparing it with a large density of minute voids.

The two drawbacks preventing the use of selective coatings on all flat-plate collectors are (1) their cost, and (2) their degradation at high operating temperatures. Degradation of absorber coatings may be caused by thermal expansion mismatch between film and substrate, leading to flexing, or by thermal migration of atoms between the film(s) and substrate.

Now, amorphous silicon produced by r.f. glow discharge decomposition of silane adheres very strongly to both metallic and ceramic substrates, because the plasma is an effective surface scourer. However its band gap is rather far from the optimum absorption cut-off of ~ 0.6 eV ($2\ \mu\text{m}$). Amorphous silicon films prepared by electron-beam evaporation in a vacuum chamber have such a high density of defect states within the bandgap that they will absorb energies further into the infrared. On balance it seems preferable to use glow discharge amorphous silicon, which can be made with a low free-carrier density (high electrical resistivity) and low 'gap states' density, and so use its low infrared emittance. Note, however, that amorphous semiconductors may be alloyed comparatively easily to tailor their optical properties. In this case, the solar absorptance of amorphous silicon may be improved by alloying it with boron¹⁷. It is expected that films of $\text{Si}_{1-x}\text{B}_x$ prepared by the glow discharge decomposition of SiH_4 and B_2H_6 may yield solar absorptances of up to 0.91 and infrared emittances of <0.09 even at the elevated temperatures reached by solar collectors.

At temperatures above $\sim 400^\circ\text{C}$, amorphous silicon may begin to recrystallise with some loss of the desired properties.

Significant changes should only occur after many years of collector use at this temperature. A second possible problem is that at these temperatures (which may be reached by concentrator collectors) hydrogen may be evolved from the film. Hydrogen evolution tends to decrease the bandgap¹⁸, which is not necessarily undesirable. Annealing of amorphous silicon films (that is prolonged periods at high temperatures) also seems to reduce the intensity of infrared absorption bands near $5\ \mu\text{m}$ (which are due to Si-H stretch modes), and so should reduce infrared emittance. This may prove to be a situation in which ageing is beneficial.

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articles

Characterisation of dielectric loss in solids and liquids

Robert M. Hill

Chelsea College, University of London, Pulton Place, London SW6, UK

The measured dielectric properties of a range of solids and liquids, as a function of frequency, have been examined and the loss has been shown to fit a generalised empirical relationship. This includes the Cole-Cole, Fuoss-Kirkwood, Cole-Davidson and Williams-Watts functions as particular cases and implies that a more generalised approach to the dielectric properties of materials is required than that given by the conventional distribution function extension of Debye's method, or by the dipole autocorrelation function of Williams and Watts.

OUR present understanding of the dielectric properties of materials is based on the Debye theory of a relaxing dipole interacting with an applied electric field¹. Early work showed that a set of exactly equivalent, non-interacting, dipoles characterised by a single relaxation time adequately explained the behaviour of weak dipolar solutions or dipolar molecules in the gas phase, but was insufficient to account for the broader frequency range over which dispersion was observed in solids and liquids in the frequency range below $\sim 10^{10}$ Hz. This difficulty was circumvented by the consideration of distributions of relaxation times² or by dipole autocorrelation³ in these more interactive media. These approaches have led to the situation where experimental measurements are analysed in terms of the

degree of fit to Cole–Cole, Cole–Davidson, Fuoss–Kirkwood, Williams–Watts or other empirical functions.

The results of a critical examination of the frequency dependence of the permittivity in a number of dielectric materials are reported here and compared with these standard functions. It is shown that the overall pattern of behaviour is broader than can be characterised by any one particular function and an empirical, but quite general, dielectric loss relationship is proposed and used to characterise the observed behaviour. The analytical method is based on the technique of temperature reduced variables⁴ with both logarithmic permittivity and frequency. The latter is common but the former is equally applicable and Jonscher⁵ has already shown that for frequencies greater than

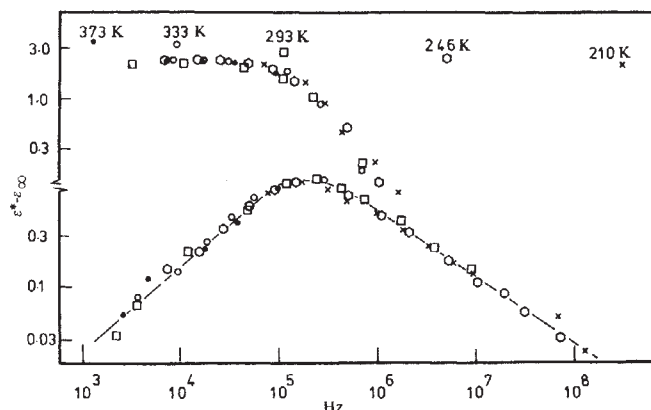


Fig. 1 The real and imaginary components of the permittivity of pentachlorotoluene⁷ as a function of frequency. The diagram has been plotted in the form of a temperature reduced master curve to show the power law characteristics at frequencies below and above that of the peak loss. The scales are correct for $T = 293$ K and the curve shifts are given by the temperature labelled datum points at the top of the diagram. The curve through the loss characteristic is given by equation (1) with the parameters listed in Table 1.

the loss peak value, ω_p , both the real and imaginary components of the complex permittivity are proportional to $\omega^{-(1-n)}$, where ω is the frequency of measurement and n lies in the range between zero and unity.

By forming a master curve from the data over suitable temperature ranges the accuracy with which the pattern of permittivity behaviour, as a function of frequency, could be determined has been increased. In all cases clear evidence of power law behaviour with ϵ'' proportional to $\omega^{-(1-n)}$ for $\omega > \omega_p$ and proportional to ω^m for $\omega < \omega_p$ has been observed, a general relationship that is masked in the more common linear permittivity/logarithmic frequency plots⁶. Several empirical functions with these power relationships as limiting cases, were investigated and it has been found that the relationship

$$\epsilon'' \propto \frac{\omega^m}{(\omega_p^{2s} + \omega^{2s})^{(m+1-n)/2s}} \quad (1)$$

gave remarkably good agreement with the experimentally determined plots. The parameter s dominates in the region of the peak only, that is $\omega \approx \omega_p$, and determines the curvature between the decaying power characteristics, low values of s giving a broad, flat, peak in the loss. The parameters m , n and s all lie in the range between zero and unity.

Equation (1) obviously includes Jonscher's relationship at high frequencies. For the particular case of $m = (1-n) = s = 1.0$ equation (1) degenerates to the Debye case, and with $m = (1-n) \neq 1.0$ Cole–Cole and Fuoss–Kirkwood plots can be derived, as can the Cole–Davidson characteristic when $m = 1.0$ and in this case $\beta_{CD} = (1-n)$. The Williams–Watts plots of ϵ'' as a function of frequency³ also show the power characteristics but

in this case there is no simple relationship between β_{WW} and either of the gradients m and $(1-n)$ although increasing values of β_{WW} gives increasing values of both gradients.

Examination of experimental data

Published loss data for a range of dielectric materials, each over a set of temperatures, was plotted logarithmically on both permittivity and frequency axes. A single master curve was then constructed by pure translation, in both axes, for each temperature. The choice of axial shift was arbitrary, but minimal scatter in the reduced data points was sought. In a few cases both the real and the imaginary components of the permittivity were available and, as the translation is identical for each of these, this made the choice of axial shift more certain. Along with the experimental points a datum from the original plot was marked to give the magnitude of the translations. Note that the tracing technique gives the inverse of the temperature dependence for both the permittivity and frequency. The final master curve

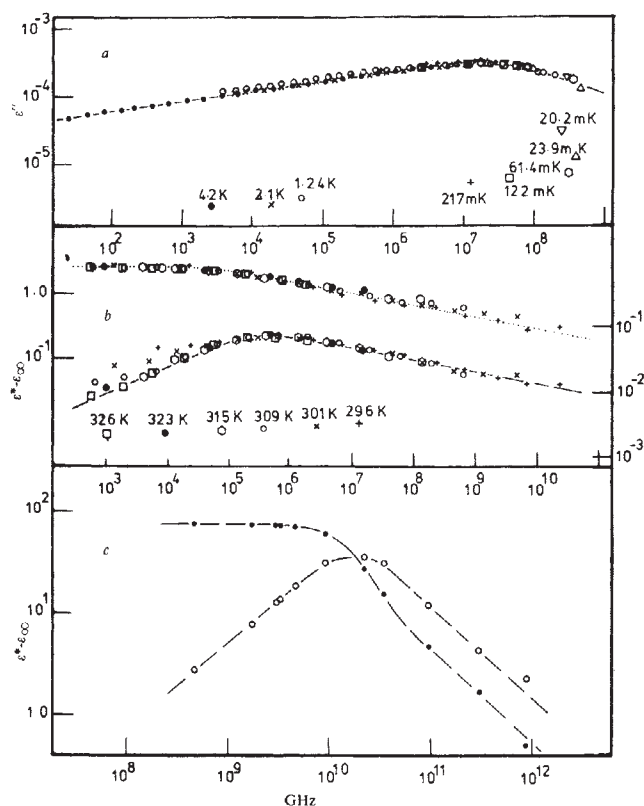


Fig. 2 Temperature reduced plots of the permittivity of *a*, suprasil glass (J. le G. Gilchrist, personal communication) (scaled at 4.2 K); *b*, poly- γ -benzyl-L-glutamate⁸ (scaled at 326 K); *c*, water at 293 K (ref. 9). In all cases the curves through the loss peaks are given by equation (1) with the parameters listed in Table 1. The suprasil glass shows the frequency extent of the low frequency power characteristic. In (*b*) the dotted curve through the real part of the permittivity is a Kramers–Kronig transformation of the loss characteristic. The measured gradients of the loss characteristics for water are 0.963 and -0.968 indicative of a Cole–Cole process, but the specimen is clearly not Debye-like as the real component of the permittivity does not have a slope of -2.0 but obeys the Jonscher relationship in being parallel to the loss at frequencies greater than that of the loss peak.

contains only the information that was originally available but presented in a form that is more easily analysed, as the superimposition of a number of equivalent measurements reduces the experimental inaccuracies. In all cases the master plot showed a characteristic loss peak with linear wings, clearly indicative of the power law characteristics exhibited by equation

(1). The gradients m and $-(1-n)$ were determined and the parameter s chosen to give a good fit of the empirical relationship to the master curve in the region of the loss peak. In a few cases, where the real part of the permittivity was available, a Kramers-Kronig transform of the loss relationship, equation (1), was computed and excellent agreement with the experimental data was obtained for the real part of the permittivity.

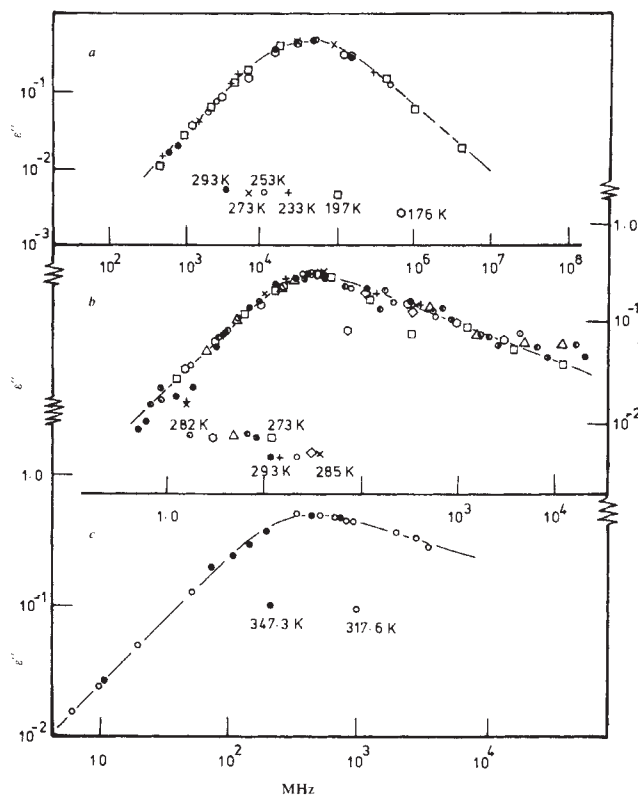


Fig. 3 Temperature reduced plots of the permittivity of *a*, 2,4,6-tri-*tert*-butylphenol¹² (scaled at 293 K); *b*, butyl stearate¹⁴ (scaled at 273 K); *c*, liquid menthol¹³ (scaled at 317.6 K). All three specimens exhibit a low frequency slope of 1.0 and the curves through the data points are given by equation (1) with the parameters listed in Table 1. This behaviour is equivalent to a Cole-Davidson distribution of relaxation times.

Figure 1 shows the reduced plot for both the real and imaginary relative permittivity for pentachlorotoluene from the data given by Turney⁷. Jonscher's power relationship for frequencies greater than the peak with $(1-n)=0.64$ is clearly shown in the loss curve but the real component has merged with the infinite frequency value and could not be determined with any accuracy. The low frequency power relationship, with $m=0.87$ is well defined and the curve through the loss data points is the relevant plot of equation (1) with s equal to unity.

The extent of the low frequency relationship is shown by Gilchrist's measurements on Suprasil glass at very low temperatures (personal communication), Fig. 2*a*. In the reduced plot, six decades of frequency exhibit the characteristic, and again the degenerate form of equation (1), $s=1.0$, applies. The fully developed proportionality between the real and imaginary parts of the permittivity for poly- γ -benzyl-L-glutamate⁸ is shown in Fig. 2*b*. In this plot, as in Fig. 1, the real and imaginary components have been displaced arbitrarily on the permittivity axis for clarity. The ratio of the absolute values of the permittivities at high, reduced, frequencies shows the predicted $\tan[(1-n)\pi/2]$ relationship⁵. At low temperatures the low, reduced, frequency data show deviation from the power characteristic due to an increasing distortion from a second, lower

Table 1 Dielectric properties

Material	m	$1-n$	s	Plot no.	Refs
Pentachlorotoluene	0.87	0.64	1.0	1	7
Suprasil glass, $T \leq 4.2$ K	0.15	0.31	1.0	2	*
Poly- γ -benzyl-L-glutamate	0.42	0.19	0.9	3	8
Water at 293 K	0.96(3)	0.96(8)	1.0	4	9
2:4:6 Tri- <i>tert</i> -butylphenol	1.0	0.66	1.0	5	12
Menthol (liquid)	1.0	0.22	1.0	6	13
Butyl stearate, $f > 10^9$ Hz	1.0	0.34	1.0	7	14

* J. le G. Gilchrist, personal communication

frequency, loss peak, the frequency separation between the two peaks decreasing as the temperature is lowered. The best fit master curve for the loss data required that s should be 0.9 ± 0.03 . The other parameters are listed in Table 1. In Fig. 2*b* as in Fig. 1, the temperature data lie on a straight line showing that there is a power relationship between the temperature dependence of the frequency shift, which is included in the term ω_p , the peak frequency, and the temperature dependence of the magnitude of the permittivity.

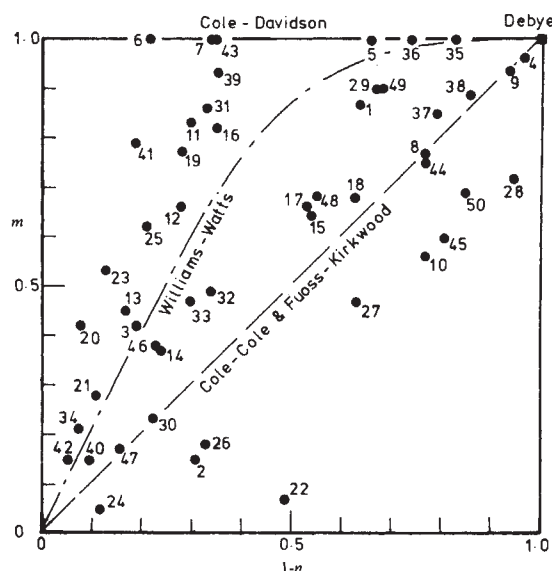


Fig. 4 A plot of the gradients m and $(1-n)$ determined for 50 loss peaks covering a wide range of polymeric and non-polymeric solids and liquids. Superimposed on the plot are the regions of validity of the Cole-Cole, Cole-Davidson, Fuoss-Kirkwood and Williams-Watts functions. Tables 1 and 2 list the gradient values.

None of these three plots show Debye, Cole-Cole or Cole-Davidson behaviour. The closest that has been observed to either Debye or Cole-Cole are the selected values of Mason *et al.*⁹ for water at 20 °C, which are presented in Fig. 2*c*. The deviation from equality in the magnitudes of the slopes, $m=0.963$, $(1-n)=0.968$, is within the accuracy of the determination of the slopes. The figure, however, shows a clear divergence from Debye behaviour in that the Jonscher relationship at high frequencies excludes the ω^{-2} decrease in the real part of the permittivity. Only if the loss decays exactly as the inverse of the frequency can the square law decrease be observed. The line through the loss data is, again, the empirical relationship. Ice at 181 K (ref. 10) and solid D₂O (ref. 11) have been observed to show similar equality in the gradients in log/log master plots and their characteristic parameters are given in Table 2.

Table 2 Master plots and characteristics

Material	Plot no.	Refs
H ₂ O at 181 K	8	10
D ₂ O, $T \leq 262.3$ K	9	11
Butyl stearate, $f < 10^9$ Hz	10	14
Poly- <i>n</i> -butyl methacrylate	11	15
Polymethyl methacrylate	12	6
Polymethyl methacrylate	13	15
Polypropylene	14	16
Amorphous polyacetaldehyde	15	17
Polyethylene, $4.2 \leq T < 18$ K	16	*
Oxidised high density polyethylene, $T < 4.2$ K	17	*
Deuterated high density polyethylene, $T < 4.2$ K	18	*
Polyethylene terephthalate		
α	19	18
β	20	18
Polyvinyl fluoride	21	19
Polyvinylidene fluoride		
α	22	20
β	23	20
γ	24	20
Polydian carbonate	25	21
Polyacronitrile	26	22
<i>n</i> -docosyl bromide	27	23
Neo-hexanol	28	24
5 methyl—3 heptanol		
α	29	25
β	30	25
3 methyl—3 heptanol		
α	31	25
β	32	25
Cyclohexyl chloride in polystyrene		
α	33	26
β	34	26
Tricyclohexyl carbinol,		
$f > 10^9$ Hz	35	27
$f < 10^9$ Hz	36	27
Picric acid	37	28
Pinacol hydrate	38	29
Chlorobenzene-pyridine, 43.4%		
α	39	30
β	40	30
Chlorobenzene- <i>cis</i> -decalin,		
α	41	25
β	42	25
Benzene, $f > 10^9$ Hz	43	31
<i>P</i> -methoxyphenylazoxy- <i>p</i> '-butylbenzene,		
nematic phase	44	32
isotropic phase	45	32
Polychloroprene		
α	46	33
β	47	33
Ba Ti ₆ MgO ₁₆	48	34
AgNa(NO ₂) ₂	49	35
Natural quartz containing impurity ions	50	36

* J. le G. Gilchrist, personal communication

Cole–Davidson behaviour, $m = 1.0$, has been observed in a number of materials, particularly when the loss peak has been observed in the frequency range from 10^7 to 10^{10} Hz. Figure 3 shows three sets of data with this characteristic, all of them from this frequency range, and all of them showing agreement with the empirical relationship. The characteristic parameters of these, and several other materials, are given in Table 1.

Some 40 materials have been examined using the technique described above and as some of these showed both α and β loss peaks about 50 loss characteristics have been determined. Figure 4 summarises the accumulated data in the form of a plot of m against $(1 - n)$. For clarity the points have been labelled and Tables 1 and 2 give a key to the data. It is apparent that the

distribution is not random but tends to cluster above the diagonal line $m = (1 - n)$. As well as the characteristic points the plot also shows the region of validity of the standard distribution and autocorrelation functions. The Debye characteristic is a single point, and is indicated by a square at (1, 1). The Cole–Davidson function requires that m should be unity and hence is given by the line along the top of Fig. 4, whereas the symmetry of the Cole–Cole and Fuoss–Kirkwood functions requires that data exhibiting this characteristic should lie on the diagonal from (0, 0) to (1, 1). The Williams–Watts expression is more complex, exhibiting a gradient of two for small values of β_{ww} and then smoothly merging with the upper limit for larger values of β_{ww} . The plot shows that although a small number of loss peaks lie on these characteristics the general distribution is much broader than these specific cases and that a more general approach to the nature of dielectric loss is required.

Conclusions

Our understanding of the dielectric behaviour in solids and liquids based on specific distributions of relaxation times or autocorrelation functions seems to be unsatisfactory. The force-fitting of experimental data into two or three types of behaviour is physically unfounded in view of the information presented in Fig. 4. The empirical loss characteristic proposed, equation (1), does not, at present, yield any physical understanding of the processes involved but has been shown to describe the experimental observations satisfactorily. The necessity for three parameters is clear as the loss curves are characterised by two power laws and a region of variable curvature. An empirical distribution function containing m , $(1 - n)$ and s could be generated but would contain no more understanding of the processes leading to loss than the empirical loss function itself. Rather, the function can be used as a test for new, more generalised, theories. One approach resulting in an expression for dielectric loss of the form of equation (1) will be presented in a later paper. Correlation of the parameters m , $(1 - n)$ and s , and of the temperature dependence of the frequency and permittivity datum shifts with the physical properties of the solid and liquid materials should begin to yield valid information on the precise nature of the interaction of electric fields and real materials.

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Bedforms and their hydraulic stability relationships in a tidal environment, Bay of Fundy, Canada

Robert W. Dalrymple*

Department of Geology and Mineralogy, Oxford University, Parks Road, Oxford, UK

R. John Knight

Petro-Canada Exploration Inc., 650 Guinness House, 727-7th Avenue SW, Calgary, Alberta, Canada T2P 0Z6

Joseph J. Lambiase

Department of Geological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Three intermediate- to large-scale bed configurations are recognised (from intertidal sand bodies in the Bay of Fundy), each with a discrete hydraulic stability field. Type 1 megaripples ('bars') form at lower flow velocities than Type 2 megaripples ('dunes'), whereas Type 2 megaripples and megarippled sandwaves are separated primarily by grain size. Megarippled sandwaves occur only in sands coarser than 0.308 mm.

TRANSVERSE sedimentary bedforms are produced by currents in many aqueous environments, and are of great interest to engineers and geologists. Because the complex nature of sediment-fluid interactions has prevented the development of a complete, theoretical understanding of bedform mechanics, attention has focused on empirical attempts to determine the hydraulic conditions governing bedform occurrence. Scaled-down flume experiments with steady-flow conditions have been used extensively¹⁻⁶. The results of such studies have greatly increased our knowledge of bedforms, but the much larger scale of most natural flows, and the unsteady conditions characterising them, limit the applicability of flume data to the real world. Complimentary bedform and hydraulic data from natural environments are needed.

This article presents a large body of such data, collected from intertidal sand bodies in the Bay of Fundy, Canada (Fig. 1). Bedform characteristics were recorded at approximately 760 locations and current velocity variations were monitored over complete tidal cycles at 110 stations. Nearly 3,000 vertical velocity profiles were measured. These data indicate that bedform development in this natural environment is more complex than that observed in flumes.

The study area

The investigations reported here⁷⁻⁹ were conducted in the eastern arm of the Bay of Fundy, on large intertidal sand bodies that occur in Cobequid Bay, the Avon River estuary, and along the north shore of the Minas Basin. The tides here are semidiurnal, anomalistic, and large, with a maximum measured range of 16.3 m at Burntcoat Head^{10,11}, and a mean range¹² of 11.9 m. Maximum water depths at high tide vary from 5 to 20 m, but depths at the times of peak current speed are generally between 2 and 10 m. The rate of water level rise and fall commonly exceeds 2 m h⁻¹.

Mean tidal current speeds ($\bar{U}$) vary greatly during each tidal cycle, and between neap and spring tides. The maximum speeds reach values of 0.5–2.0 m s⁻¹ during the middle to late part of the ebb tide, and in the first half of the flood. Cor-

responding maximum shear velocities fall between 0.03 and 0.15 m s⁻¹. The duration of peak flow strengths ranges from 1 to 4 h. The flow directions of the ebb and flood currents are generally 180° apart, but inequalities in the magnitude and/or duration of the opposing currents produce a persistent residual transport of sediment at most localities. Thus, either the ebb or flood portion of the tidal cycle dominates bedform formation and migration at any site.

The sediments on the sand bodies have mean sizes of 1.6–0.125 mm (–0.7 to 3.0 phi: very coarse to fine sand), but most are medium sand (0.5–0.25 mm: 1.0–2.0 phi). These sands exhibit good sorting, with standard deviations that are generally less than 0.8 phi units.

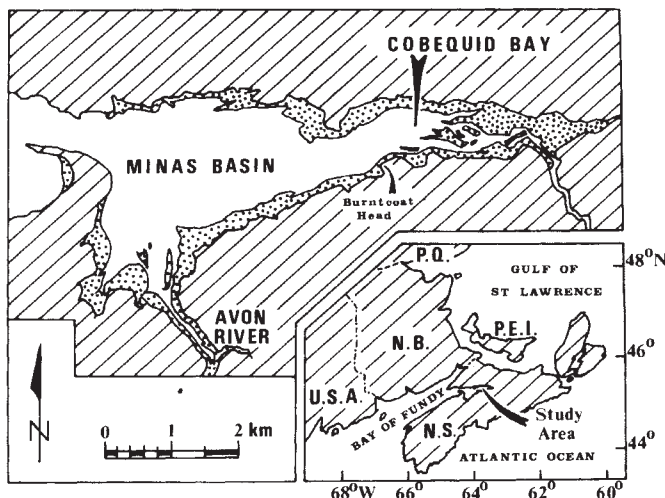
Additional information concerning the studied areas can be found in refs 7–9 and 13–16.

Bedform morphologies

Lower flow regime bedforms^{1,2} are abundantly developed on the sand bodies, and three basic types are recognised on the basis of size and morphology—ripples, megaripples, and sandwaves (Table 1). Upper flow regime plane bed^{1,2} is observed to occur during peak flow at only two locations in the Avon River estuary⁹.

Because of the great changes in flow direction, strength, and depth that occur during a tidal cycle, various modifications of the basic bedform types are produced^{7-9,13}, including: planing-off of megaripple crests in shallow flows; incomplete reversal of

Fig. 1 Map of the study area showing its location in the eastern arm of the Bay of Fundy (insert), and the extent of the intertidal zone at spring low tide (stippled pattern).



* Present address: Department of Geological Sciences, Brock University, St. Catharines, Ontario, Canada L2S 3A1.

Table 1 Summary of bedform size and morphological characteristics

Bedform type	Wavelength (<i>L</i>) (m)	Height (<i>H</i>) (m)	Steepness (<i>L/H</i>)	Morphological characteristics
Small scale: Ripples	<0.3	<0.05	~10	Straight to linguoid in plan Usually superimposed on larger forms as a late-stage modification
Intermediate scale: Type 1 Megaripples	0.1–25.0 (6.1)	0.05–0.50 (0.18)	10–150 (44.6)	Straight to smoothly sinuous in plan, without small sinuous irregularities Lack scour pits Height remains constant along crestline Flattened in section (<i>L/H</i> usually >20) Wavelengths and heights poorly correlated ($r = 0.462$ for $n = 70$), with a best-fit regression line of $H = 0.0947 (L)^{0.346}$ Less faces at angle of response, producing tabular cross bedding
Type 2 Megaripples	0.05–14.0 (4.3)	0.05–0.70 (0.28)	6–34 (16.5)	Generally sinuous to lunate in plan, but may be straight with small sinuous irregularities Scour pits usually well developed Height variable along crestline Profiles are steep (<i>L/H</i> usually <20) Wavelengths and heights well correlated ($r = 0.788$ for $n = 255$), with a best-fit regression line of $H = 0.0865 (L)^{0.787}$ Less faces at angle of repose, producing trough cross bedding
Large scale: Megarippled sandwaves	10.0–215.0 (40.6)	0.15–3.4 (1.86)	17–210 (44.1)	Straight to smoothly sinuous in plan Scour pits absent Height constant along crestline Lee face inclination generally 10°–20° Wavelengths and heights moderately correlated ($r = 0.791$ for $n = 58$), with a best-fit regression line of $H = 0.0635 (L)^{0.733}$ Megaripples (usually Type 2, but less commonly Type 1) are superimposed on the sandwaves
Rippled sandwaves	5.0–25.0 (12.9)	0.15–0.75 (0.38)	30–55 (36.6)	Morphologically similar to megarippled sandwaves but only have ripples or lower flow regime plane bed superimposed on their stoss sides

Average values of height, wavelength and steepness index (*L/H*) are given in parentheses. r = correlation coefficient; n = no. of data points.

megaripples; and superposition of current ripples on all surfaces (Fig. 2) during the decelerating stages of the ebb tide. To simplify the analysis, and make the results more directly comparable with other environments, these modifications are not considered here.

Ripples are the smallest bedform type (Table 1) with heights and wavelengths of less than 0.05 m and 0.3 m respectively¹⁷. All shapes from straight to linguoid are observed. Ripples most commonly occur superimposed on the larger bedforms, but they are found on their own in a few localities. Only the latter are considered in the hydraulic section below.

Megaripples¹⁸ (Fig. 2*a, b*) are analogous to the dunes of most authors^{1,17,19}, and are intermediate in scale between ripples and sandwaves (Table 1). All megaripples have a lee face that is at the angle of repose of the sediment during active migration, but otherwise show a wide variation in detailed morphology. We have, however, arbitrarily subdivided them into two varieties—Type 1 and Type 2 megaripples. No single characteristic can be used to distinguish between the two types, and a continuous gradation occurs between them.

Type 1 megaripples are simple in appearance, and are characterised by straight to smoothly sinuous lee faces that lack irregularities with a wavelength of ~2–3 m (Fig. 2*a*). Scour pits are not present in the troughs: consequently, lateral continuity is good, and the height is very regular along an individual bedform. The wavelengths of these megaripples are long relative to the height, giving them flat profiles with wavelength/height ratios generally greater than 20 (Fig. 3). In addition, the heights and wavelengths of Type 1 megaripples are poorly correlated (Fig. 3, Table 1).

Type 2 megaripples are more complex in appearance (Fig. 2*b*), and are generally sinuous to lunate in plan. Some straight-crested examples do occur, but these exhibit small, sinuous irregularities along the lee face with a wavelength ~2–3 m. The troughs contain well-developed scour pits (Fig. 2*b*), commonly separated by prominent spurs. As a result, bedform height varies considerably over short distances along the crestline. Type 2 megaripples have steep profiles, and on average they are higher and shorter than Type 1 megaripples (Fig. 3, Table 1). The heights and wavelengths of Type 2 megaripples are quite well correlated (Table 1).

Sandwaves^{13,14,20,21} are the largest scale of bedform (Fig. 2*c*), with average heights and wavelengths markedly larger than those of megaripples (Fig. 3, Table 1). Most sandwaves are asymmetrical, but their lee faces commonly have inclinations of only 10–20°. (This is believed to be a result of the reversing flows, as possible fluvial counterparts possess avalanche faces^{22,23}.) Sandwaves display many similarities to Type 1 megaripples: they are straight to smoothly sinuous in plan; their troughs lack scour pits; the height remains uniform along a particular bedform (Fig. 2*c*); and they have flattened profiles with wavelength/height ratios rarely less than 20 (Fig. 3); but the heights and wavelengths of sandwaves are moderately well correlated (Table 1). Complete morphological gradations are observed between Type 1 megaripples and sandwaves, but no such gradational relationship is seen between sandwaves and Type 2 megaripples.

Virtually all sandwaves bear megaripples (Type 2 forms generally) on their stoss sides, producing 'megarippled sandwaves' (Fig. 2*c*). These megaripples do not appear to be a

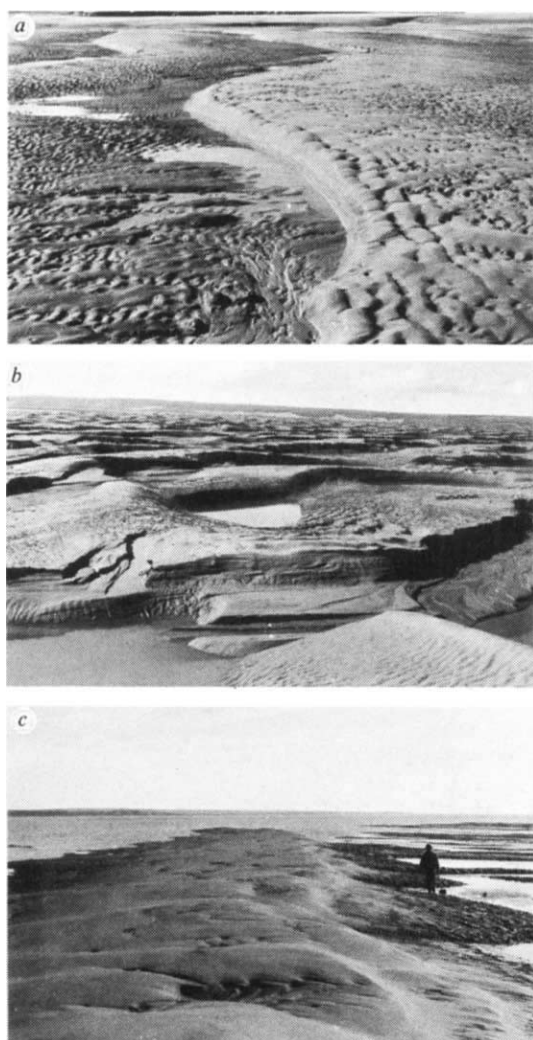


Fig. 2 Intermediate- and large-scale bedform types. *a*, Type 1 megaripples: note the simple plan geometry, absence of scour pits, and uniformity of bedform height (~ 0.2 m). *b*, Type 2 megaripples with well-developed scour pits and three-dimensional geometries. Metre stick at right for scale. *c*, Megarippled sandwaves: note the straight crestline and the lack of scour pits, other than those associated with the superimposed Type 2 megaripples. The megaripples on the sandwave crest have been partially rounded-off by wave action during emergence.

late-stage modification as are the superimposed current ripples. Instead, echo-sounder records show⁸ that the megaripples are present and active throughout both the semidiurnal and neap-spring tidal cycles at most localities. This megaripple-on-sandwave superposition seems, therefore, to represent an equilibrium co-existence, comparable to examples reported from both fluvial²² and shallow marine²⁴ settings.

In a few instances, only ripples or lower flow regime plane beds are present on the sandwave stoss sides. These forms, called rippled sandwaves, are smaller on average than megarippled sandwaves (Table 1), but are otherwise similar in morphology. Observations suggest that rippled sandwaves may be a disequilibrium form of megarippled sandwaves, because the former almost always occur in situations of decelerating flow: either as a neap-tide replacement of megarippled sandwaves that exist during spring tides; or as a transitional form between megarippled sandwaves and Type 1 megaripples, produced by bedform migration into an area of lower current strength and shallower water depth. In both cases, the superimposed megaripples disappear before the sandwave because of their smaller size and shorter lag time^{25,26}. During the prograde transition from Type 1 megaripples to mega-

rippled sandwaves, a gradual increase in bedform size commences at the same time as the superimposed megaripples appear, and rippled sandwaves do not occur as an obvious intermediate step. These observations may support experimental work suggesting that bedform destruction is a less 'efficient' and slower process than bedform generation²⁷.

Hydraulic relationships

Bedforms respond in some manner to all flows that are above the threshold of sediment movement, but lag behind changes in flow conditions because of the finite time required to reach a new equilibrium^{25,26}. As a result, it is difficult to determine a specific set of hydraulic conditions responsible for the bedforms observed in the study area, because flow conditions vary greatly over the semidiurnal tidal cycle. It is thought, however, that a reasonable approximation to these 'effective' conditions is obtained by assuming that the major bedforms are produced by the peak flow during the dominant half of the tidal cycle. This is the period when the largest amount of sediment is transported, and data⁸ on the variation of friction factors over the tidal cycle suggest that the bedforms approach equilibrium most closely at this time.

Therefore, representative 'average-maximum' values of the mean current speed ($\bar{U}$) have been calculated by averaging the three highest mean speeds measured in water depths greater than 2.5 m during the dominant half of each monitored tidal cycle. (The depth limit of 2.5 m was established because fast currents in shallower flows were observed to plane-off pre-existing bedform crests.) Average-maximum values of shear velocity (U_*) and stream power (ω) also were calculated in the same manner. The mean water depth was taken as the average of the depths in which the three maximum $\bar{U}$ values occurred. All of these data are summarised in Table 2, and phase diagrams involving water depth, mean current speed and grain size are shown in Fig. 4.

Figure 4 shows that a good separation of the bedform types is achieved using the above procedure. Some overlap does occur, but this is to be expected because of the complexity of the environment as compared to closely controlled flume experiments. One factor not previously considered that contributes to the overlap in terms of $\bar{U}$ is the variation of flow strength with tidal range. During neap and spring tides, the maximum current speeds may, for a few tidal cycles, fall outside the stability range of the bed configuration developed by the preceding average tides. Because of lag effects^{25,26}, the bedforms do not always achieve a new equilibrium, and thus exist 'metastably'

Fig. 3 Plot of bedform height (H) against wavelength (L). Envelopes to the observed values are given for Type 1 megaripples, Type 2 megaripples and megarippled sandwaves. The thin diagonal lines are contours of the steepness index (L/H). $\circ$, Type 1 megaripples; $\bullet$, Type 2 megaripples; $\square$, rippled sandwaves; $\blacksquare$, megarippled sandwaves.

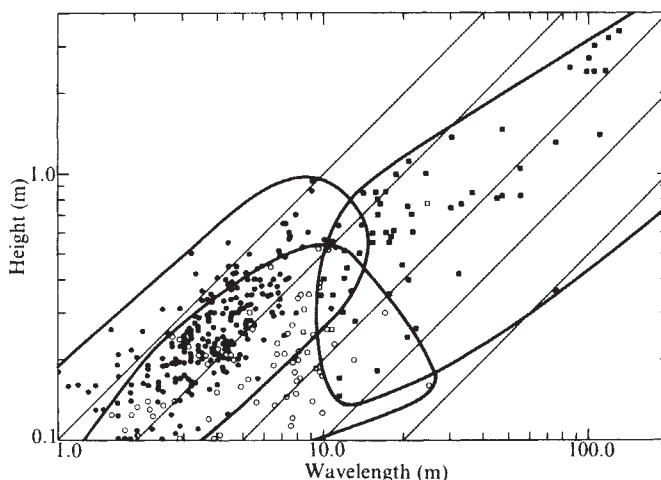
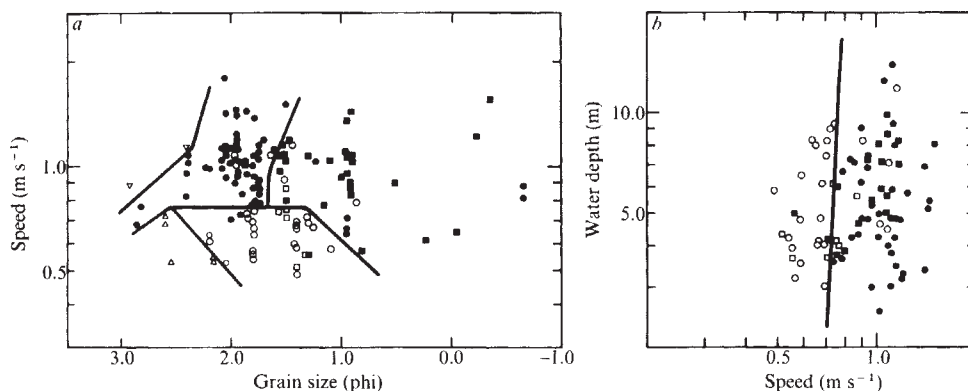


Fig. 4 Bedform phase diagrams, constructed by the method outlined in the text. The dominant half of the tidal cycle was established by comparing average-maximum values of $\bar{U}$ for the ebb and flood. The bed configuration developed at each site was determined by direct observation during emergence and/or from echo-sounder records. Δ , Ripples; $\circ$, Type 1 megaripples; $\bullet$, Type 2 megaripples; $\square$, rippled sandwaves; $\blacksquare$, megarippled sandwaves; ∇ , upper flow regime plane bed. *a*, Average-maximum mean current speed plotted against mean grain size. Data for all water depths are included. *b*, Mean water depth plotted against average-maximum mean speed. Only data for medium sand (0.5–0.25 mm: 1.0–2.0 phi) are included.



out of their normal stability field, thereby creating overlap. However, this does not obscure the basic hydraulic relationships.

In the study area, ripples occur alone, without larger bedforms, only where the mean sediment size is <0.25 mm (2.0 phi) and the average-maximum current speed is below 0.75 m s^{-1} (Fig. 4a). The upper flow regime plane bed field also is restricted to fine grain size (less than ~ 0.21 mm: 2.25 phi) within the range of velocities encountered.

Type 1 megaripples form in sands coarser than 0.18 mm (2.5 phi) at average-maximum mean speeds comparable to those producing ripples, and at flow strengths (as expressed by either $\bar{U}$, u_* , or ω) which are considerably below those producing Type 2 megaripples (Fig. 4a, Table 2). This flow-strength distinction between straight-crested (Type 1) and three-dimensional (Type 2) megaripples has been known qualitatively for some time¹⁷, but has not previously been quantified. The degree of separation of the two megaripple types in Fig. 4 is remarkable, given the gradational nature of the morphological transition between them. This indicates that the distinction used here is not only consistent, but also of potential genetic significance.

Megarippled sandwaves also form at higher flow strengths than Type 1 megaripples, but at a slightly lower average strength than Type 2 megaripples (Fig. 4a, Table 2). However, the overlap between megarippled sandwaves and Type 2 megaripples in terms of $\bar{U}$ (and also u_* and ω) is large (Fig. 4); and both bedform types form in an almost identical range of water depths (Fig. 4b), a finding which sharply disagrees with the results of a previous study²⁸. Clearly, mean grain size is the only major variable that distinguishes between these two bed configurations (Fig. 4a): sandwaves are restricted to sediments coarser than approximately 0.308 mm (1.70 phi); whereas

megaripples coexist with sandwaves at those grain sizes but occur alone in finer sands. (Some Type 2 megaripple points plot within the megarippled sandwave stability field in Fig. 4a, but most of these represent starved megaripples migrating over gravel lag or bedrock. It may be that sandwaves, like desert draas²⁹, require a minimum thickness of mobile sediment before their formation is possible.)

Rippled sandwaves have not been given a separate stability field in Fig. 4a and b, because they overlap considerably with Type 1 megaripples and to a lesser extent with megarippled sandwaves. This behaviour agrees with the earlier suggestion that rippled sandwaves are a retrograde form of megarippled sandwaves.

Discussion

Comparison of the current speed-grain size phase diagram developed for this natural environment (Fig. 4a) with those constructed from shallow (0.2–0.4 m) flume experiments^{6,30,31} shows a close similarity in the positioning and slope of the various phase boundaries, despite the vastly different flow depths. The flume studies reveal that the stability field of dunes gradually pinches out as the sediment size decreases, so that dunes do not occur at grain sizes finer than approximately 0.1 mm (3.3 phi)³⁰. If Type 1 and Type 2 megaripples are considered together, then a similar pattern is evident in Fig. 4a. In addition, the slope of the Type 1 megaripple phase boundaries in Fig. 4a matches that of 'sand waves' in ref. 31 over the corresponding range of grain sizes.

These similarities, plus the near-vertical orientation of the Type 1–Type 2 megaripple boundary in Fig. 4b, are clear evidence that water depth has little influence on bedform development, especially in flow depths as great as those encountered in the study area.

Table 2 Mean values of sediment and flow parameters characterising the occurrence of the various bedform types

Bedform type	Mean grain size (mm)	Average-maximum mean speed (m s^{-1})	Mean water depth (m)	Average-maximum shear velocity (m s^{-1})	Average-maximum stream power ($\text{kg s}^{-1} \text{m}^{-1}$)
Ripples	0.184 (2.44 ϕ)	0.603	5.37	0.035	0.739
Type 1 Megaripples	0.328 (1.61 ϕ)	0.687	5.57	0.044	1.800
Type 2 Megaripples	0.272 (1.88 ϕ)	1.052	6.00	0.082	7.628
Megarippled sandwaves	0.507 (0.98 ϕ)	0.955	6.45	0.065	4.748
Rippled sandwaves	0.369 (1.44 ϕ)	0.701	4.50	0.045	1.750

Examination of the hydraulic and morphological data indicates that Type 1 megaripples are equivalent to the 'bars' described by Costello⁶, whereas Type 2 megaripples represent the dunes of refs 6, 30, and 31. Confusion arises, however, because 'bars' are, unfortunately, also called 'sand waves', and are thought^{6,30,31} to be a smaller-scale equivalent of the large sandwaves reported from many natural environments^{13,20-22}. The positioning of the distinct megarippled sandwave field in Fig. 4a does not seem to support such a comparison, despite the many morphological similarities between sandwaves and Type 1 megaripples ('bars').

It has been suggested^{32,33} that sandwaves are large dunes, which are in equilibrium with deeper and faster flows than smaller dunes. The large overlap of the megarippled sandwave and Type 2 megaripple stability fields in terms of both speed and depth (Fig. 4a, b) strongly indicates that this interpretation is not valid, at least in the study area. The formation of megaripples and sandwaves in similar water depths (Fig. 4b, Table 2) also calls into question the generality of the commonly cited relationship between water depth and bedform wavelength³⁴, which forms the basis of the computational model of bedform lag developed recently by Allen^{33,35}. The results of such models must, therefore, be treated with caution until more is known about sandwaves and their genetic relationship to 'bars' and dunes. Clearly, further detailed study in natural environments is necessary until such time as sandwaves can be reproduced in flumes.

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Transformation of yeast by a replicating hybrid plasmid

Jean D. Beggs

Department of Molecular Biology, University of Edinburgh, Edinburgh, UK and Department of Cytogenetics, Plant Breeding Institute, Trumpington, Cambridge, UK

Chimaeric plasmids have been constructed containing a yeast plasmid and fragments of yeast nuclear DNA linked to pMB9, a derivative of the ColEI plasmid from E. coli. Two plasmids were isolated which complement leuB mutations in E. coli. These plasmids have been used to develop a method for transforming a leu2 strain of S. cerevisiae to Leu⁺ with high frequency. The yeast transformants contained multiple plasmid copies which were recovered by transformation in E. coli. The yeast plasmid sequence recombined intramolecularly during propagation in yeast.

PROCEDURES for cloning DNA segments in *Escherichia coli* by insertion into a plasmid or bacteriophage genome are now well established and have been used to isolate many prokaryotic and eukaryotic DNA sequences. However, many eukaryotic genes do not have counterparts in bacteria and therefore cannot be isolated by complementation of a bacterial deficiency, and

sequences involved in regulation or development may only be usefully studied in a homologous genetic background.

A yeast cloning system would be extremely useful for the isolation of genes and regulatory sequences from yeast and other eukaryotic organisms and for the genetic manipulation of commercially important yeasts. Recently yeast cells were transformed at a frequency of 10^{-7} transformants per viable cell using a derivative of the bacterial plasmid ColEI carrying the yeast *LEU2* gene which codes for β -isopropylmalate dehydrogenase¹. The transforming plasmid was apparently not present in yeast clones in the free or non-integrated form but was integrated into yeast chromosomes at more than one site.

I describe here the development of a highly efficient yeast transformation system using yeast-bacterial hybrid plasmids which are able to replicate and may be selected in and recovered from both *E. coli* and *Saccharomyces cerevisiae*. The hybrid plasmids incorporate a yeast plasmid, 2 μ m long (approximately 6 kilobases)²⁻⁵ which was previously cloned in bacteriophage λ and analysed by restriction endonuclease digestion to determine its suitability as a cloning vehicle⁶.

The yeast plasmid is present in many strains of *S. cerevisiae*

Fig. 1 Plasmid pMB9, the 2- μ m plasmids from *S. cerevisiae*, and the hybrid plasmids used to clone nuclear DNA fragments from *S. cerevisiae*. The physical map of pMB9 is essentially that described by Bojivar *et al.*¹⁵ and the *HpaI* endonuclease target was mapped in this work. The tetracycline resistance gene is represented by Tc^r. The *S. cerevisiae* plasmid types A and B which are presumably interconvertible are represented as dumbbells with the inverted repeat regions corresponding to the stem portion. The sizes of *EcoRI* restriction digest fragments are given as kilobases and were reported previously⁶. The structures are shown of the four hybrid plasmids chosen for further cloning experiments out of the eight possible configurations. To construct these hybrid plasmids, purified¹⁶ yeast plasmid DNA was restricted with *EcoRI* endonuclease¹⁹ under conditions such that digestion was incomplete and the majority of the products were in the form of full length linear molecules as determined by agarose gel electrophoresis¹⁹. Plasmid pMB9 DNA purified from chloramphenicol-treated cultures²⁰ by a cleared lysate procedure²¹ was digested with *EcoRI* endonuclease and the two digested DNA samples were mixed and ligated²². *E. coli* strain ED8767 (*recA56 metB⁻ hsdS⁻ supE⁻ supF⁻*; W. J. Brammar) grown in L-broth²² was made competent by calcium chloride starvation²³ and transformed by heat shock treatment with DNA at 0.3 μ g ml⁻¹. Cells were diluted threefold with L-broth and incubated at 37 °C for 40 min before plating to select for transformants on fresh L-broth agar containing tetracycline hydrochloride at 15 μ g ml⁻¹. Transformant clones were screened for the presence of the yeast plasmid sequence by *in situ* hybridisation²⁴ with a ³²P-labelled probe prepared by nick translation²⁵ of DNA from a lambda clone of the yeast plasmid⁶. Clones of plasmids containing the yeast sequence were further analysed to identify those in which the complete yeast plasmid was present. The size of plasmid in each clone was estimated by gel electrophoresis of colony lysates²⁶. Clones with full-size hybrid plasmids were further characterised by restriction analysis of the plasmid DNA.

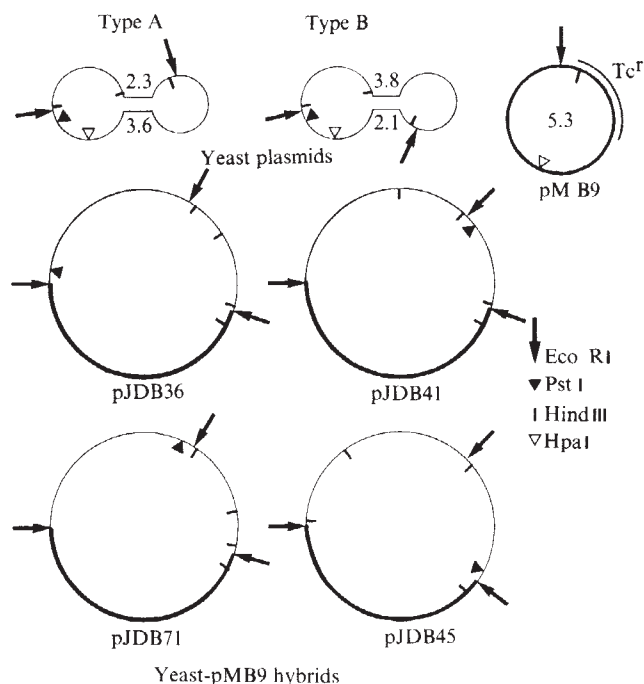
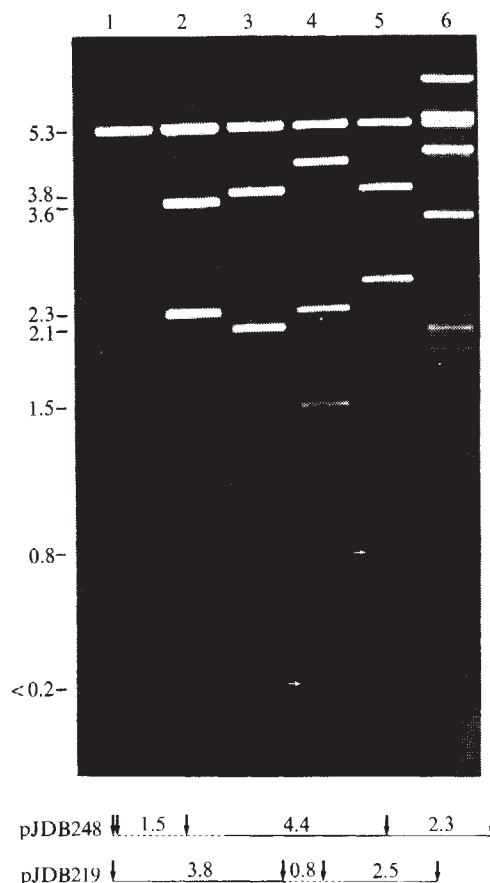


Fig. 2 Cloning of *leuB*-complementing sequences from yeast nuclear DNA into hybrid plasmids. *EcoRI* endonuclease digests of (1) pMB9 DNA, (2) pJDB36 (type A hybrid plasmid) DNA, (3) pJDB41 (type B hybrid plasmid) DNA, (4) pJDB248 (type A *leu⁺* plasmid), (5) pJDB219 (type B *leu⁺* plasmid) and (6) *EcoRI* endonuclease digest of λ DNA mixed with a *HpaI* endonuclease digest of λ DNA. DNA fragment sizes are given in kilobases and faint bands are indicated by arrows. In the diagram only yeast sequences in pJDB248 and pJDB219 are shown. The yeast plasmid sequence is represented by a continuous line and the yeast nuclear DNA inserts by discontinuous lines. Arrows in the diagram represent sites for *EcoRI* endonuclease. Nuclear DNA (a gift from C. Christiansen) from *S. cerevisiae* strain M127 (prototrophic) was sheared in a Virtis 60K homogeniser²⁷ such that the major fraction of sheared DNA was in fragments 6 to 15 kilobases long with a smaller proportion of fragments of length ranging to less than 1 kilobase. A mixture of the DNAs of the hybrid plasmids pJDB36, pJDB41, pJDB45 and pJDB71 was digested with *PstI* endonuclease. Both the yeast and the plasmid DNAs were extracted with phenol, extracted with ether and extensively dialysed against 0.1 M potassium cacodylate pH 7.0. Poly(dA) tails were added to the yeast DNA and poly(dT) tails were added to the plasmid DNAs by incubation with calf thymus terminal deoxynucleotidyl transferase. The reaction conditions were as described by Lobban and Kaiser²⁸, except that the temperature of the poly(dT) reaction was lowered to 0 °C, and in both reactions MgCl₂ was replaced by 0.5 mM CoCl₂ (ref. 29). The reactions were stopped by the addition of EDTA when 50 nucleotides had been incorporated per 3' terminus. The tailed DNAs (0.37 μ g of each DNA ml⁻¹) were annealed in 10 mM Tris, 1 mM EDTA, 100 mM NaCl pH 8 by incubating at 65 °C for 10 min and allowing to cool to room temperature in the same water bath. The DNA was cloned in *E. coli* JA221 by selection of tetracycline-resistant transformants. A total of 21,000 clones were stored in batches of 500 at -80 °C in 12.3 g per l K₂HPO₄, 0.9 g per l sodium citrate, 0.18 g per l MgSO₄·7H₂O, 1.8 g per l (NH₄)₂SO₄, 3.6 g per l KH₂PO₄, 88 g per l glycerol (D. Hogness, personal communication). In addition 5,000 of these clones were maintained individually in L-broth containing 7% dimethylsulphoxide in microtitre plates at -20 °C. Clones with plasmids complementing the *leuB6* mutation in *E. coli* JA221 were selected by growth of samples from the batches of clones on minimal selective plates (the minimal medium of Spizizen³⁰ was used with 0.2% w/v glucose, 1.5% Difco Bacto agar and 20 μ g ml⁻¹ L-tryptophan). The *leuB6*-complementing plasmids (pJDB219 and pJDB248) were analysed by digestion of the DNAs with *EcoRI*, *HindIII*, and *HpaI* endonucleases and gel electrophoresis to determine the configuration of the hybrid plasmid in each case. Electrophoresis was through a 1% agarose horizontal slab gel (18 × 24 × 0.5 cm) in 40 mM Tris, 20 mM sodium acetate, 1 mM EDTA pH 8.2 at 100 V per 30 mA for 15 h. The gel was stained by soaking in a solution of ethidium bromide (0.5 μ g ml⁻¹) and photographed with a UV light source below.



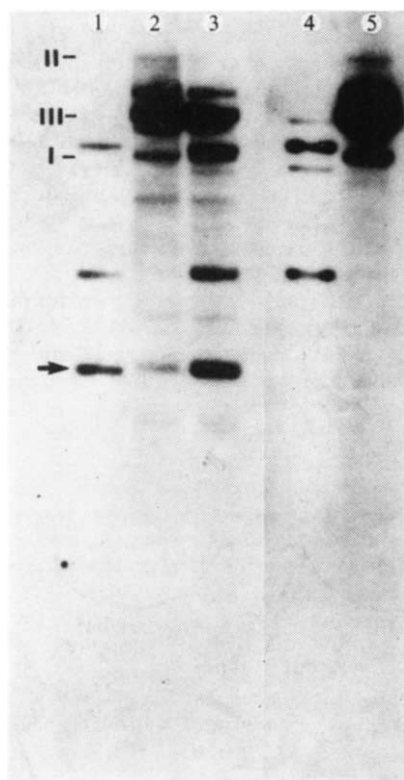


Fig. 3 Autoradiograph of ^{32}P -labelled cRNA prepared³¹ to *EcoRI* digested DNA of pJDB248 (1, 2 and 3) or pJDB36 (4 and 5) hybridised with *EcoRI* digested pJDB248 DNA (1 and 4), *S. cerevisiae* DNA (2 and 5) or *S. cerevisiae* and pJDB248 DNAs together (3). The bands corresponding to the 1.5 kilobase DNA fragment are indicated by an arrow. The slower migrating bands (I, II, III) observed in the yeast DNA digests correspond to the supercoiled, nicked-circular and linear forms respectively of 2- μm yeast plasmid which was relatively resistant to digestion by *EcoRI* endonuclease although there were two targets for this enzyme in the plasmid sequence of this strain. Digestion of plasmid DNA with *EcoRI* endonuclease before the preparation of cRNA had the effect of increasing the efficiency of the RNA polymerase reaction, but the yeast sequences were then preferentially transcribed, resulting in the relatively lower intensity of the band due to pMB9 on the autoradiograph. The *S. cerevisiae* DNA used in this experiment was a different preparation from that used in the cloning experiment (and was prepared as described in the legend to Fig. 5). The possibility that the cloned DNA fragment was a contaminant in the DNA preparation is therefore unlikely. *EcoRI* digested DNAs were electrophoresed through a 1.5% agarose slab gel at 120 V per 45 mA for 13 h and transferred³² to a nitrocellulose filter (Millipore HAWP). Hybridisation of the filters was carried out at 60 °C in 4 ml 2 $\times$ SSC (0.03 M sodium citrate, 0.3 M sodium chloride, pH 7.0) containing 20 μg *E. coli* tRNA and cRNA (300,000 d.p.m.) with rapid shaking for 16 h. The filters were washed in 1 $\times$ SSC at 60 °C with gentle shaking for 2 h. The sensitivity of Kodak X-Omat H film was enhanced by using Ilford fast tungstate screens and storing at -80 °C during the exposure³⁵.

but has no known function. The plasmid has the same density as main band nuclear DNA^{2,5} and replicates under nuclear control^{7,8}, there being 50 to 100 plasmid copies per cell⁹. It contains an inverted repeat sequence 600 base pairs long and is isolated as a mixture of two forms which differ from one another in the orientation of the two nonrepeated segments of the molecule with respect to each other. The two forms are presumably due to recombination between the inverted repeat sequences¹⁰⁻¹². Plasmids isolated from several different strains of *S. cerevisiae* differ from each other by small deletions or point mutations which alter the restriction enzyme cleavage pattern of the DNAs^{13,14}.

Construction of chimaeric plasmids

The plasmids were constructed in two stages. First, pMB9, a derivative of the ColEI plasmid from *E. coli*¹⁵, and the plasmid from *S. cerevisiae* DR19/8T¹⁶ were joined by ligation of the DNA fragments produced by *EcoRI* endonuclease (Fig. 1). The pMB9 sequence includes a tetracycline resistance determinant and tetracycline-resistant clones were selected after transforming *E. coli* with the ligated DNAs. These clones were screened for a hybrid plasmid large enough to carry the complete yeast plasmid sequence.

Hybrid yeast-pMB9 plasmids theoretically have eight possible configurations. These are determined by whether the yeast sequence is in type A or type B configuration, by which of the two *EcoRI* endonuclease targets joins the sequence to pMB9, and by the orientation of insertion into pMB9. These configurations can be differentiated by the digestion pattern of the plasmid DNA with *HindIII* or *HpaI* endonucleases. Five of these different configurations were found amongst seven complete hybrid plasmids examined. The significance of the two forms (A and B) of yeast plasmid was not known. Nor was it known whether cloning DNA fragments into either of the *EcoRI* sites of the yeast plasmid would inactivate a sequence required for the maintenance of the plasmid in yeast or for some other essential function. Therefore, four of the different hybrid plasmids, pJDB36, pJDB41, pJDB45 and pJDB71 (Fig. 1), were used in the second stage of the construction of the plasmids to be used in the transformation of yeast.

Sheared nuclear DNA from *S. cerevisiae* of average fragment length 6 to 15 kilobases was linked by means of poly(dA-dT) tails¹⁷ to a mixture of DNAs of pJDB36, pJDB41, pJDB45 and pJDB71 which had been converted to linear molecules by digestion with *PstI* endonuclease. Tetracycline resistant clones were selected after transformation of *E. coli* JA221 (*recA1*, *leuB6*, *trp Δ E5*, *hsdR⁻* *hsdM⁺* *lacY* C600; J. Carbon) with the annealed DNAs. A total of 21,000 clones were obtained, and those which were phenotypically *leu⁺* were isolated by selection on minimal plates. Two plasmids, pJDB219 and pJDB248, were isolated which contained a sequence able to complement the *leuB6* mutation in strains JA221 and C600. Both plasmids transformed strain JA221 to *leu⁺* or to tetracycline resistance with equal efficiency (10^5 transformants per μg DNA). The plasmids also complemented the *leuB61* and *leuB401* alleles¹⁸ at the same frequency as transformation to tetracycline resistance in the same strains.

Plasmid pJDB248 was pJDB36 with an insertion of approximately 2.4 kilobases containing two sites for *EcoRI* endonuclease which were 1.5 kilobases apart (Fig. 2). Plasmid pJDB219 was formed by an insertion of approximately 1.2 kilobases (containing one target for *EcoRI* endonuclease) in pJDB41.

Minor bands corresponding to DNA sequences of 3.8 and 2.9 kilobases were observed in varying amounts in *EcoRI* digests of different preparations of pJDB248 DNA from cultures produced from purified colonies. These corresponded to the fragment sizes expected if pJDB248 contained the type B yeast plasmid sequence rather than type A. Presumably the yeast inverted repeat sequences recombined during propagation in *E. coli*. This recombination was not observed in other *E. coli*-yeast hybrid plasmids. However, the rate of recombination may be slow relative to the growth rate of *E. coli*, and the growth of cultures from original clones was not extended beyond the minimum required for preparation of plasmid DNA.

Figure 3 shows that DNA from *S. cerevisiae* digested with *EcoRI* endonuclease contained a 1.5 kilobase sequence which hybridised to the ^{32}P -labelled cRNA prepared to pJDB248 DNA, but not to cRNA prepared to pJDB36. This indicates that the inserted sequence corresponds to a yeast DNA fragment. The same *EcoRI*-generated DNA fragment hybridised cRNA prepared to pJDB219, indicating that the *leuB*-complementing sequences in the two independently isolated plasmids were the same.

Transformation of yeast

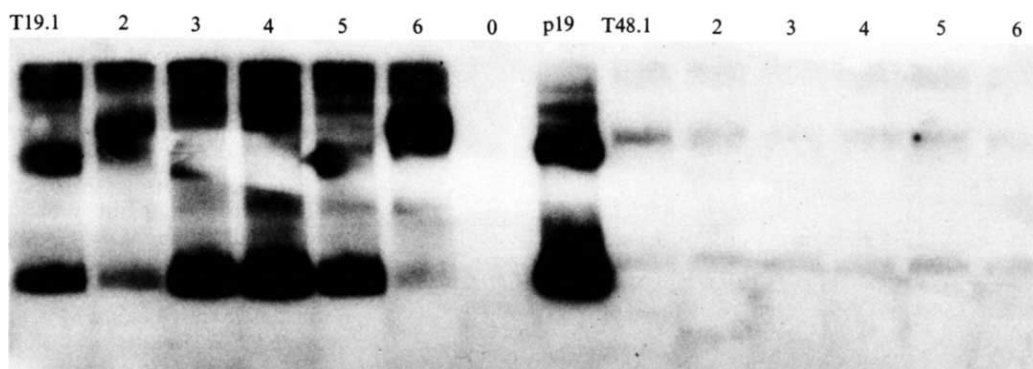
S. cerevisiae MC16 α leu2-3 his4-712^{FS} ade2-1 lys2-1 SUF2; G. R. Fink (FS denotes a suppressible mutation, SUF2 is a suppressor gene) was used as the recipient strain to investigate the uptake and replication of the plasmids and the transfer of the Leu⁺ phenotype. Protoplasts and DNA were mixed and treated with polyethylene glycol to promote DNA uptake (see legend to Fig. 4). In different experiments between 10% and 30% of the cells thus treated regenerated the cell wall and produced colonies in supplemented minimal agar. Viable protoplasts were transformed to LEU⁺ by pJDB219 or pJDB248 DNA with a frequency of 5×10^{-4} to 3×10^{-3} transformants per viable cell. The *leu2* mutation in this strain reverted to LEU⁺ at a frequency of only 10^{-8} to 10^{-7} . Clones transformed by pJDB248 grew as fast on selective plates as the untransformed cells grew on supplemented plates: protoplasts gave rise to colonies in 2 to 3 days. Transformants of pJDB219 required 3 to 4 days to develop colonies. [Note, efficiency of transformation of *S. cerevisiae* was not linearly proportional to DNA concentration, and a saturating DNA concentration was not found. Transformation frequencies of 10^4 to 10^5 transformants per μ g DNA were obtained in the conditions used here.]

To detect the presence of the transforming plasmids in yeast clones, extracts of yeast cells were analysed by gel electrophoresis, transfer of the DNA to nitrocellulose and hybridisation with a ³²P-labelled cRNA prepared to pMB9 DNA. Transformants T19.1 to T19.6 (Fig. 4) produced bands hybridising to the pMB9 sequence and with similar electrophoretic mobility to the transforming plasmid pJDB219 (upper and lower bands correspond to nicked circular and supercoiled molecules respectively, slower migrating minor bands probably represent

oligomeric forms of the plasmid). Transformants T48.1 to T48.6 also contained plasmids with the pMB9 sequence and with lower electrophoretic mobility than pJDB219, as expected for the larger plasmid pJDB248 (not shown in Fig. 4). There were apparently more copies per cell of pJDB219 than of pJDB248 in their respective transformant clones.

To investigate the integrity of the plasmid sequence in transformants, DNA was extracted and analysed by digestion with *Eco*RI endonuclease. Figure 5 shows the banding pattern of *Eco*RI-digested plasmid DNA isolated from two yeast clones transformed by pJDB219. The bands found in digests of pJDB219 DNA (corresponding to 5.3, 3.8, 2.5 and 0.8 kilobase DNA fragments) can be seen in the transformant DNA digests, and also the bands found in the digests of 2- μ m plasmid DNA (3.8, 3.6, 2.3 and 2.1 kilobase fragments). There is also a band in the transformant DNAs corresponding to a 4.0 kilobase fragment which would be expected if the yeast plasmid in pJDB219 recombined during propagation in yeast, converting a type B to a type A plasmid (fragments of length 3.8 and 2.5 kilobases would be replaced by fragments of length 4.0 and 2.3 kilobases). Thus all the bands in the plasmid DNA of one transformant (Fig. 5, track 2) are those expected for a mixture of the 2 μ m plasmid and pJDB219, however, two more bands can be seen in the plasmid DNA of the other transformant (Fig. 5, track 3) corresponding to 3.4 and 1.9 kilobase fragments. These bands cannot be readily accounted for. They may be products of recombination between plasmids¹¹, or of specific deletion events. Two examples of each type of banding pattern were found in digests of DNA from four different yeast clones transformed by pJDB219. The DNA from three yeast clones transformed by pJDB248 DNA was similarly analysed. The

Fig. 4 Autoradiograph of ³²P-labelled cRNA to pMB9 DNA hybridised with undigested DNA of lysates of yeast clones transformed with pJDB219 (clones T19.1 to T19.6) or pJDB248 (clones T48.1 to T48.6) and in the lysate of the untransformed recipient strain alone (0) and with pJDB219 DNA (p19). Cultures of *S. cerevisiae* MC16 to be transformed were grown on YPDA



(1% yeast extract, 2% Difco bacto-peptone, 2% glucose, 20 mg l⁻¹ adenine) and collected at 10^8 cells ml⁻¹. Cells were washed in sterile distilled water, concentrated threefold in 1.2 M sorbitol, 25 mM EDTA, 50 mM dithiothreitol pH 8 and incubated at 29°C for 10 min with gentle shaking. Cells were washed twice by centrifugation and resuspension in the original culture volume of cold, sterile 1.2 M sorbitol. Protoplasts were obtained by concentrating the cells threefold in 1.2 M sorbitol, 0.01 M EDTA, 0.1 M sodium citrate pH 5.8 containing Helicase lyophilised snail gut extract at 2 mg ml⁻¹ (Industrie Biologique Francaise; 2 g powder containing 10^6 units β -glucuronidase activity were dissolved in 10 ml distilled water, filter sterilised and stored at -20°C), and incubated at 29°C with gentle shaking for 1 h. Protoplasts were washed by centrifugation and resuspension in the original culture volume of 1.2 M sorbitol three times to remove nuclease activities present in the snail gut extract, and resuspended in 1.2 M sorbitol 10 mM CaCl₂ to give approximately 10^{10} protoplasts ml⁻¹. DNA was mixed with 0.1 ml or 0.2 ml protoplasts to give 10 to 20 μ g DNA ml⁻¹ (DNA samples were sterilised immediately before use by extraction with chloroform and extraction with ether). Protoplasts and DNA were left at room temperature for 15 min and then diluted with 10 volumes of 20% polyethylene glycol 4000, 10 mM CaCl₂, 10 mM Tris-HCl pH 7.5 (a modification of a protoplast fusion procedure³³). After 15 to 20 min protoplasts were pelleted and resuspended in 100 μ l 1.2 M sorbitol, 10 mM CaCl₂, 20 μ g ml⁻¹ leucine plus 50 μ l YPDA containing 1.2 M sorbitol, and incubated at 29°C for 20 min. Samples were diluted in 1.2 M sorbitol and plated by mixing in 7 ml minimal medium containing 1.2 M sorbitol and 3% agar at 45°C (minimal medium was 0.67% Difco yeast nitrogen base without amino acids, 2% glucose, 20 mg l⁻¹ adenine, lysine and histidine) and pouring onto minimal medium plates containing 1.2 M sorbitol, 2% agar. Dilutions were plated on supplemented (20 μ g leucine ml⁻¹) minimal plates to measure the efficiency of protoplast regeneration. Colonies developed after 2 to 4 days incubation at 29°C. To test for the presence of the transforming plasmid, single clones were purified on minimal selective plates. Single colonies were used as inocula to make 1 cm $\times$ 2 cm streaks on YPDA plates which were incubated at 29°C overnight. These streaks of cells were resuspended in 0.5 ml 1.2 M sorbitol, 25 mM EDTA, 0.86 M 2-mercaptoethanol, pH 8, left at room temperature for 10 to 20 min and centrifuged. The pelleted cells were resuspended in 0.5 ml 1.2 M sorbitol, 0.1 M sodium citrate, 10 mM EDTA pH 5.8 containing Helicase snail gut extract at 2 mg ml⁻¹ and incubated at 29°C for 1.5 h. Protoplasts were washed twice by centrifugation and resuspension in 1.5 ml 1.2 M sorbitol and finally resuspended in 200 μ l 10 mM Tris, 1 mM EDTA, 1% sodium dodecyl sulphate pH 8. Protoplasts were lysed by incubation at 60°C for 20 min and debris was pelleted. Lysate (40 μ l) was mixed with 20 μ l 20% Ficoll, 0.05% bromophenol blue and electrophoresed through 1% agarose slab gels at 120 V per 35 mA for 15 h. Gels were exposed to short wavelength ultraviolet light for 15 min to nick the supercoiled plasmids and transferred to nitrocellulose filters as usual³², except that the denaturation step was carried out at 37°C for 1 h to ensure plasmid denaturation and to hydrolyse RNA. The filters were hybridised with ³²P-labelled cRNA (300,000 d.p.m.) as described in Fig. 2.

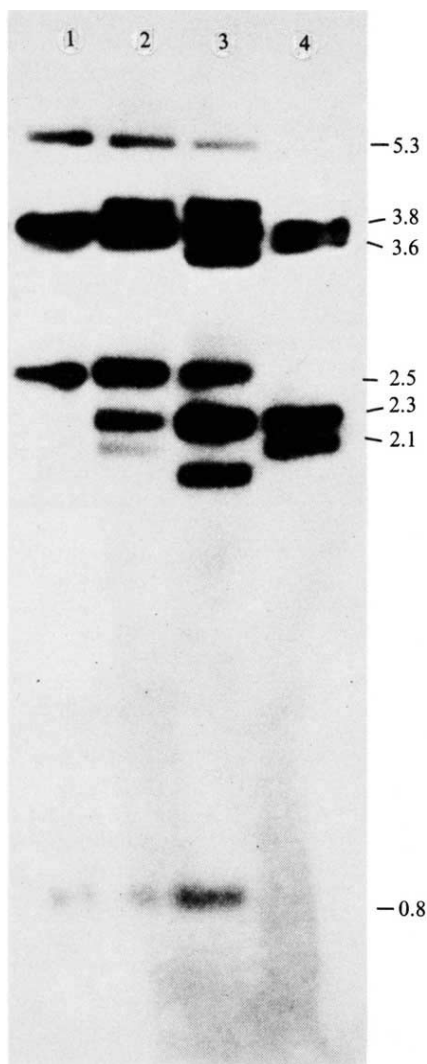


Fig. 5 Autoradiograph of ^{32}P -labelled cRNA to *Eco*RI digested DNA of pJDB219 hybridised with *Eco*RI digested DNA from yeast clones transformed by pJDB219. (1) pJDB219 DNA; (2) DNA from yeast clone T19.1 in Fig. 4; (3) DNA from yeast clone T19.2 in Fig. 4; (4) DNA from the untransformed yeast strain. DNA fragment sizes are indicated in kilobases. DNA was partially purified from cultures grown on YPGA medium by lysis of protoplasts³⁴ and extraction of the lysates with phenol and chloroform. Nucleic acids were ethanol precipitated, resuspended, and RNA was digested by pancreatic ribonuclease³⁴. The samples were finally dialysed against 5 mM Tris, 0.5 mM EDTA pH 8.

unexpected 3.4 and 1.9 kilobase fragments were produced from the DNA of two of these clones (not shown).

Recovery in *E. coli* of plasmids from yeast transformants

Samples of DNA (prepared as described in Fig. 5) from six pJDB219 transformants were used to transform *E. coli* JA221. Transformed cells were plated on minimal agar, minimal agar containing tetracycline ($15 \mu\text{g ml}^{-1}$) or leucine-supplemented agar containing tetracycline. No significant difference was found between the transformation frequencies for the *leuB* and the tetracycline resistance markers selected either independently or together. This suggests that the plasmids recovered from yeast transformants by complementation in *E. coli* had retained both the tetracycline resistance and the *leuB*-complementing sequences. This test gives no information about plasmids which may have retained the *leuB*-complementing sequence but lost

the pMB9 sequence, as the yeast plasmid probably does not replicate in *E. coli* (J. D. B., unpublished result). Similar results were obtained in recovering DNA from pJDB248 transformants although the level of complementation was reduced probably as a result of the low copy number of plasmid in these strains (see Fig. 4).

DNA of the 'recovered' pJDB219 was isolated from eight *E. coli* transformants selected by transformation to *leu*⁺ and tetracycline resistance, and from two transformants selected for tetracycline resistance alone. The *Eco*RI digestion pattern of seven of these plasmid isolates was identical to that of pJDB219 DNA. Three of the recovered plasmids contained the yeast DNA sequence in the type A rather than the type B configuration. Since pJDB219 DNA used to transform yeast was in only one configuration (type B) this confirms the theory that recombination occurs in yeast between the inverted repeat sequences of the yeast plasmid. It can also be concluded that the yeast DNA sequence in pJDB219 complements the *leuB* mutation in *E. coli* regardless of the orientation of this sequence with respect to the pMB9 sequence (the inverted repeat sequences separate pMB9 from the *leu*-complementing sequence).

The results presented in this article show that *S. cerevisiae* can be readily transformed by the hybrid plasmids and that despite the generation of unexpected recombinant plasmids in some clones the unaltered plasmid can be isolated from the yeast clones directly, or purified by recloning in *E. coli* and prepared more easily from bacterial cultures.

The inheritance of the *Leu*⁺ phenotype in the yeast clones containing pJDB219 or pJDB248 has been shown by tetrad analysis to be cytoplasmic (J. D. B. and Nasmyth, K., in preparation).

Yeast is a desirable organism to use in molecular cloning. It is readily and rapidly propagated. Its biochemical and physiological similarity to bacteria allows the use of techniques developed for bacterial systems. It offers the advantage to genetic studies of vegetative growth in both the haploid and the diploid states. The high frequency of transformation obtained with the system described here makes it feasible to isolate directly in yeast any yeast DNA sequence from a pool of hybrid plasmids carrying fragments representing the entire yeast genome.

All experiments using organisms containing recombinant plasmids were carried out under category I containment as recommended by the UK Genetic Manipulation Advisory Group. I thank Professor K. Murray and Dr R. B. Flavell for laboratory facilities and many materials, and J. F. Atkins and J. R. Bedbrook for discussions. Most enzymes were provided by K. Murray and J. R. Bedbrook. Calf thymus terminal transferase was a gift from the Corporate Lab., ICI. The work was supported in part by an SRC grant. An EMBO Short Term Fellowship was awarded to develop techniques for regenerating yeast protoplasts in the laboratory of Dr P. P. Slonimski. J. D. B. is a Beit Memorial Research Fellow.

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letters to nature

Atmospheric gravity waves generated during a solar eclipse

DURING a solar eclipse, the Moon's cool shadow, moving with supersonic speed through the Earth's atmosphere, should generate bow waves. In the experiments described here four microbarographs were used in an attempt to detect atmospheric pressure oscillations that were expected to accompany bow waves associated with the total solar eclipse of 23 October 1976. These internal gravity waves were found to have a peak-to-peak amplitude of 0.1 to 0.2 Pa, a period of 23 min and a velocity of $3.1 \times 10^2 \text{ m s}^{-1}$.

Figure 1 shows, in relation to the central line of the eclipse, the locations of the microbarographs at Tantanoola (T), Whyalla (W), Adelaide (A) and Kambalda (K), some hundreds to thousands of km apart. Their geographical coordinates are listed in Table 1. Four microbarograph records, sampled at 1-min intervals, are shown in Fig. 2. They all have dominant pressure oscillations at periods of the order of 20–25 min, with peak-to-peak amplitudes of 0.1–0.2 Pa. (Note that the microbarographs had broadband characteristics. Their maximum amplitude response was adjusted to be at a period of 6 min, which was approximately double their response to a 23-min period. It indicates that if other dominant periods had been present, these would also have been recorded.)

A microbarograph was installed at Kambalda (Western Australia) in anticipation that focusing of the bow-wave might occur in this locality due to curvature of the eclipse path and of the Earth's surface¹. In that case, a considerably greater amplitude would be expected at K compared with amplitudes at T, A and W which were well outside the expected focusing region. However, the present observations indicate similar amplitudes at all four stations, and give no suggestions of a focusing effect near K. The computation predicting focusing at K was subsequently found to be in error; S. Ball (personal communication) has shown that focusing of the bow wave should, in fact, have occurred at high northern latitudes.

At T, W and A, the weather remained fine throughout the recording time. At K, storms, accompanied by pressure fluctuations of 0.5–1.0 Pa amplitude, masked any bow wave recording except for the 2 h shown in Fig. 2.

To obtain a better estimate of the periodicity, a spectral analysis was made of each of the four records in Fig. 2. In every case, a period between 22 and 24 min was identified, the mean period being 22.7 min. This was the only periodicity common to all four records. It therefore seemed reasonable to attribute a 23-min period to a disturbance sufficiently widespread to affect all four stations, such as that generated by the eclipse.

As a further check that the 23-min period was associated with the eclipse, a comparison was made of the data shown in Fig. 3, namely the spectral components for T, which were: (1) derived for the nearly 5 h data in Fig. 2, including the eclipse interval and shortly afterwards; (2) derived for 83 min immediately before;

and (3) for 112 min immediately after the data in (1). (T was the only station for which data were available to make such a comparison.) The predominance of the 23-min period during and shortly after the eclipse, as indicated in (1), and its absence at adjacent times, confirms its association with the eclipse.

The present observation of 23 min is similar to the periodicity of about 20 min recorded^{4,5} in upper atmospheric disturbances after the eclipse of 7 March 1970.

An internal gravity wave of period 23 min should have a velocity⁶ of $3.1 \times 10^2 \text{ m s}^{-1}$. From this, and the known velocity of the eclipse 'point' along the central line of the eclipse path⁷, the positions of Q, T, S and R (called disturbance points) on the central line were estimated and are shown in Fig. 1. An eclipse-generated disturbance initiated at these points would arrive first at station K and then at T, A and W, respectively, before disturbance initiated elsewhere on the line. From the known times⁷ when the eclipse point moved through Q, R, S and T, an estimate was made of the times when the same phase of a bow wave should be recorded at K, W, A and T, respectively.

Table 1 Geographical coordinates of the four microbarographs

	Tantanoola (T)	Adelaide (A)	Whyalla (W)	Kambalda (K)
Latitude (°S)	37.7	34.8	33.0	31.2
Longitude (°E)	140.6	138.6	137.5	121.7
'Disturbance point' on central line of eclipse	T	S	R	Q
Comparative time (min) when eclipse was at dis- turbance point	20.5	17.75	15.5	0

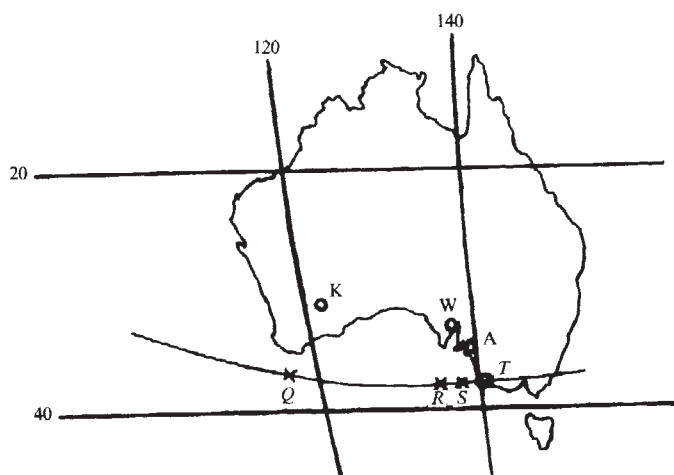


Fig. 1 Locations of the four microbarograph recording stations (T, A, W and K) and the central line of the eclipse.

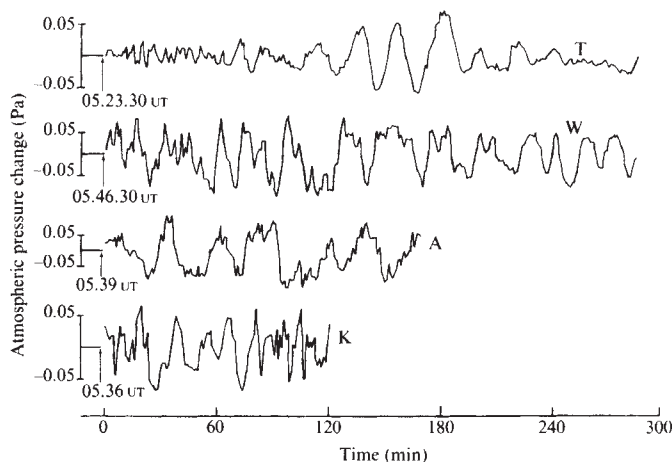
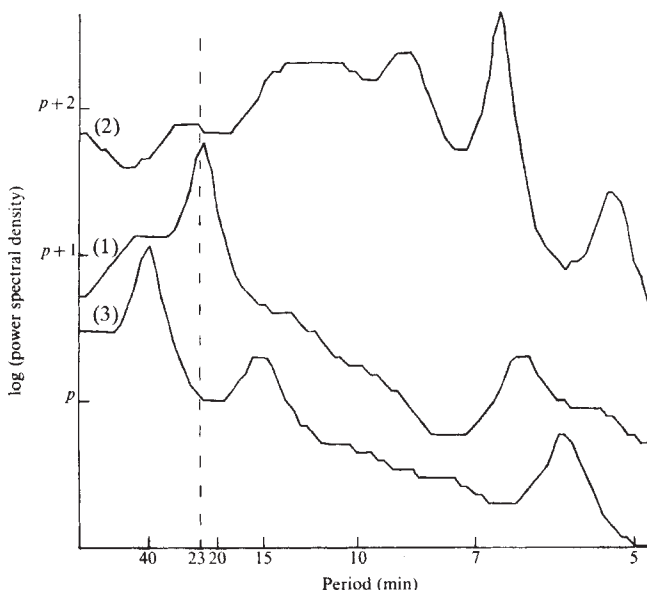


Fig. 2 Atmospheric pressure fluctuations recorded during and shortly after the eclipse at T, A, W and K.

The data were analysed further to confirm that the bow wave had the assumed velocity of $3.1 \times 10^2 \text{ m s}^{-1}$, and so to verify that an internal gravity wave was generated along the eclipse path. In the analysis, data from all stations were limited to the first 2 h plotted in Fig. 2, because useful K data were only available at that time. In estimating the bow wave velocity, it was convenient to imagine that a disturbance left Q, R, S and T simultaneously. To allow for the non-infinite eclipse velocity, an appropriate amount of time (indicated in the last row of Table 1) was subtracted from the actual times of recording for each station. The time difference between records from two stations, corrected in this way, is denoted t' . The interval, t' , between the occurrence of a particular phase of a disturbance at one station, and its occurrence at another station, is the time for a wave to travel between those stations. Knowing also the distance the wave has travelled, its velocity may be determined.

We wanted to estimate as accurately as possible the phase difference between each of the six pairs of records. To do this, cross-correlation coefficients, with time slips, were calculated for each pair of records. In Fig. 4, the cross-correlation coefficient, r , is plotted as a function of t' for the six pairs of records. (As an indication of the reliability of the data, note that the largest maximum in each of the TW and WK curves in Fig. 4 is statistically significant at the 5% significance level.)

Fig. 3 Spectral components of Tantanoola data recorded: (1) during and shortly after the eclipse; (2) immediately before data in (1); and (3) immediately after data in (1).



In principle, the value of t' at a particular correlation maximum for a pair of stations should be the time of travel of a bow wave from one station to the other, corresponding to the assumed velocity of $3.1 \times 10^2 \text{ m s}^{-1}$ ($=18.6 \text{ km min}^{-1}$). (Each curve in Fig. 4 has, in fact, several correlation peaks at approximately 23-min intervals, as expected. An acceptable correlation maximum would correspond to t' , giving a velocity of approximately the expected value.) However, the appreciable noise creates difficulty in accurately specifying the value of t' appropriate to a correlation maximum. To improve the accuracy, a sine curve of 23 min period was fitted to each cross-correlation curve. The arrows in Fig. 4 indicate when the sine curve giving best fit was a maximum, and these were taken to represent the correlation curve maxima.

The ordinate in Fig. 4 is d , the distance travelled by the bow wave from the first-mentioned station to the second. The six circles in Fig. 4 represent values of d as a function of t' , corresponding to correlation maxima. The circles for the station pairs AW, WK and AK lie close to a line of slope 18.6 km min^{-1} which passes through the origin. The observed time delays for those station pairs therefore affirm the assumed value for the bow wave velocity. The circles for the station pairs TA, TW and TK may be represented by a line of similar slope, but passing through $d = 0$ at $t' = -11 \text{ min}$. It seems significant that station T, on the central line of the eclipse, is included in all three of these latter station pairs. It suggests that the assumption of an atmospheric disturbance that occurred only on the central line of the eclipse is inadequate. In fact, the relevant width of the eclipse path was 174 km.

A cross-correlation analysis was carried out on the total T and W data in Fig. 2, extending over nearly 5 h. This gave a correlation curve maximum at $t' = 26 \text{ min}$, indicated in Fig. 4 by a square which lies close to the line of slope 18.6 km min^{-1} , passing through the origin. In contrast, the TW cross-correlation curve in Fig. 4, computed for only the first 2 h data, has a correlation maximum 11 min earlier.

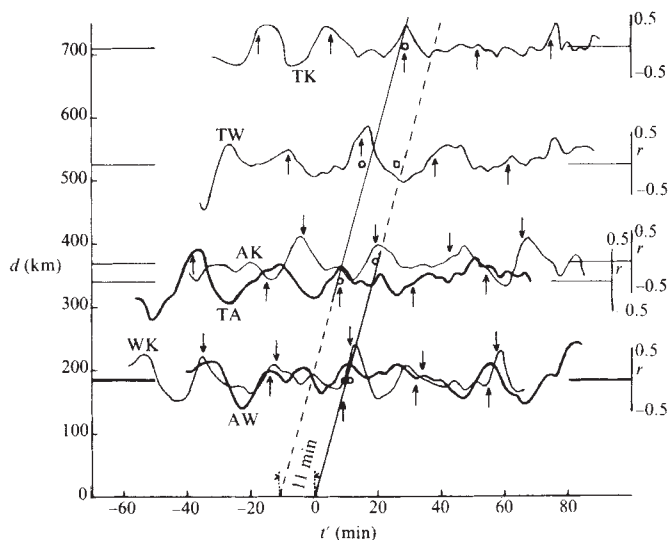


Fig. 4 Cross-correlation coefficients (r), with time slips, are shown for the six pairs of stations. t' is the time slip of the first-mentioned station of a pair ahead of the second; t' has been 'corrected' to allow for the non-infinite eclipse velocity. d is the distance travelled by a bow wave between the two stations of each pair.

Both sets of points in Fig. 4 can be represented by lines of slope 18.6 km min^{-1} , which affirms that the observed velocity was as expected for an internal gravity wave of period 23 min.

An apparent discrepancy is the additional 11 min delay observed in the early phase of the bow wave received at T, compared with that received at A, W and K. This may be explained in terms of an initial disturbance that occurred north

of the central line of the eclipse, and travelled a longer distance to T and shorter distances to A, W and K than would have been the case for a disturbance on the central line. The difference in travel times, being 11 min, would locate the actual disturbance source at, or near, the total eclipse boundary. As the data in Fig. 4 are accurate to ± 2 min, the observation could imply a source location somewhere from ground level to stratospheric heights. The point arising in the present context is that the apparent 11-min discrepancy is explainable, and that the data which involve station T are not inconsistent with other data in affirming the expected internal gravity bow wave velocity.

G. L. GOODWIN
G. J. HOBSON

School of Physics,
South Australian Institute of Technology,
Australia

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First direct measurement of the beaming of Jupiter's decametric radiation

One of the most important features of the jovian decametric radioemission comes from the geometry of the observed radiation. The emission occurrence probability appears as a well defined function of the respective positions of Jupiter, the observer and the innermost galilean satellite Io, exhibiting several major sources which correspond to narrow ranges in zenocentric observer's longitude L (the so-called central meridian longitude) and in the synodic orbital phase of Io, Φ . These statistically established directivity properties were defined by Warwick¹ and Alexander², who showed that the repetition of the distinguishable dynamic spectra associated with each source occurring in a longitude range of 20° , is attributable to a $\pm 10^\circ$ beaming cone into which the radiation escapes in the ecliptic plane. Dulk³ stated more precisely that the emission is received on the Earth when Io makes certain angles with the line of sight, and that the dynamic spectrum varies in shape and frequency range with the magnetic longitude of Io. The beaming in latitude is also important⁴: the $\pm 3.3^\circ$ variation of the zenocentric declination of the Earth is sufficient to lead to a 15° variation of the statistical apparent sub-Io longitude of the emission. But these conclusions, based on statistics and on the repeatability of similar dynamic spectra in equivalent geometrical configura-

tions of Jupiter, Io and the observer, are not decisive, as they were obtained from a single direction at a given time. We present here the first direct experimental proof of a narrow beaming of Jupiter radiation, using simultaneous observations in two different directions, during the French-Soviet space radio astronomy experiment Stereo-5.

In this experiment, the Sun was simultaneously tracked from the Earth, at Nançay Observatory, and from the Soviet spacecraft Mars 7, at the frequencies 30 and 60 MHz (ref. 5). The observations were carried out for up to two hours per day, when the Sun was near the meridian at Nançay, from August 1973 to May 1974. During this period, the spacecraft was fitted with two linear antennas perpendicular to the ecliptic plane, which also allowed us to observe Jupiter. At 30 MHz, the antenna was a full-wave dipole; taking into account its efficiency and the electrical perturbation due to the spacecraft body, its effective gain was about 4 dB in the direction of the Sun, and much less in other directions. The signals were preamplified and detected by two automatic gain-controlled receivers with 0.5 MHz bandwidth and logarithmic responses. Both outputs were digitised at 4 Hz, and data were compressed and recorded on board, before being transmitted by telemetry to the USSR and then back to France. At Nançay, the equipment consisted of two 10 dB Yagis nearly orientated towards the Sun, feeding receivers of the same kind as on the spacecraft but with a 10 Hz sampling rate.

The broadband decametric Jupiter patrol at Nançay⁶ used a linearly polarised, log-periodic antenna, with a 10 dB gain, and a swept frequency spectrograph, covering the 20–40 MHz frequency, with a 15 kHz bandwidth and a sweep rate generally close to 10 Hz; in addition, a 1 MHz multichannel spectrograph⁷, tunable in frequency, was centred at 30 MHz during the stereo periods.

We were able to compare unambiguously two pairs of simultaneous track periods, 23 March 1974 and 15 May 1974, each free of solar activity (Fig. 1). On the first day, Jupiter was recorded on the Earth and not on the probe, and vice versa on the second day. These two cases, both of which correspond to the high frequency jovian Early source, are synoptically described in Fig. 2. The configuration in the solar system is displayed in Fig. 3. Note that, when giving flux densities or times for the spacecraft, we consider what would be seen by an observer on the line Jupiter-spacecraft at a distance of Jupiter equal to the Jupiter-Earth distance. From solar burst studies, we know that the sensitivity of the Earth is about four times that on the spacecraft for a source in the direction of the antenna maximum response (neglecting any problem of polarisation). We did not expect to receive many Jupiter events from the spacecraft, owing to the amount of available observation time. At 30 MHz, it emits for only a small percentage of time. It was also, for several weeks, pointing in directions of very low spacecraft antenna gain. Moreover, the solar activity often prevented any detection of the jovian emission. Hence we did not record Jupiter's activity when the stereo angle was small.

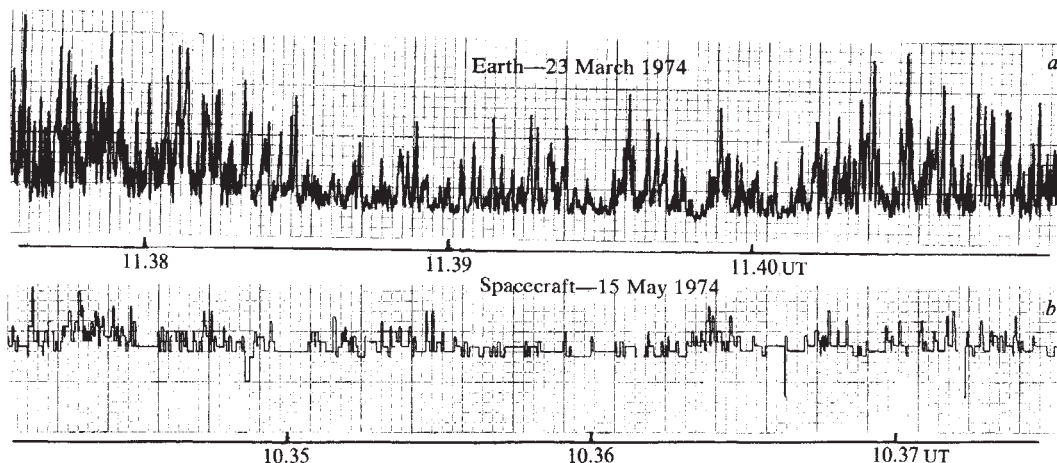


Fig. 1 Jupiter recorded at 30 MHz. On 23 March (a) the spacecraft is free of any activity; on 15 May (b) the Earth is free. The time scale for the probe is reset for a distance to Jupiter equal to that of the Earth at the same time.

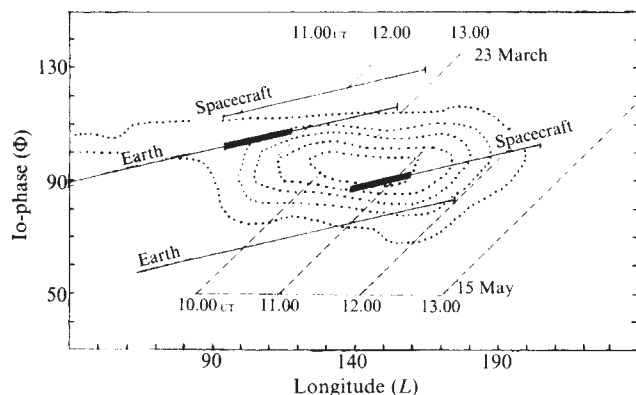
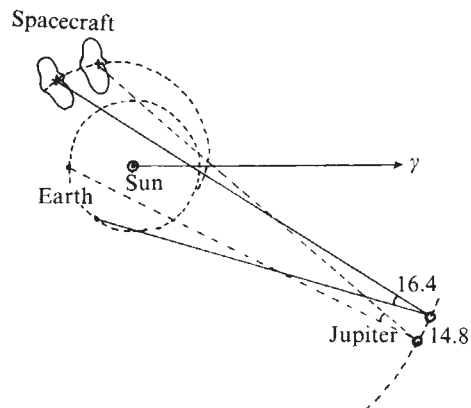


Fig. 2 Synoptic diagram of the Stereo observations of 23 March ($\theta = 14.8^\circ$) and 15 May ($\theta = 16.4^\circ$). Light lines correspond to the periods of observations; heavy lines sketch the observed jovian activity periods; dashes indicate, for both days, the UT on Earth and on the spacecraft, taking into account the delay due to propagation; dotted lines are the occurrence probability contours, as drawn from 7 to 41 MHz (University of Colorado's data).

Nevertheless, all the features of the emission recorded by the spacecraft on 15 May 1974 are typical of Jupiter activity. The emission is absent at 60 MHz and present at 30 MHz, where it reaches a maximum flux density about $8 \times 10^{-21} \text{ W m}^{-2} \text{ Hz}^{-1}$. It was recorded for half an hour; it consisted of a succession of bursts, which look like the well known *L*-bursts, with a pseudo period of the order of 2 min, exhibiting strong intensity fluctuations on a very short time scale (the solar bursts never have so short a time scale). These fluctuations might correspond either to the interplanetary scintillation or to jovian *S*-bursts, averaged by the time detection constant of the spacecraft receiver. Of course, the slower modulation (*L*-bursts) is not an ionospheric effect, as is usually assumed from the ground, but is characteristic of the emission itself and probably corresponds to drifting spectral structures in the time-frequency plane.

At that time, in Nançay, Jupiter was not recorded either by the 30 MHz receivers or at any frequency between 25 and 38 MHz by the swept frequency receiver. At the end of the observation period, the planet was at a very large zenith angle in Nançay; but it was also tracked by the University of Colorado's spectrograph, between 7 and 41 MHz, and no emission was observed⁸ from 09.00 UT until 12.00 UT, above the sensitivity threshold $5 \times 10^{-22} \text{ W m}^{-2} \text{ Hz}^{-1}$. We can estimate that, during the whole ground observation period, there was no emission more intense than one-tenth of the intensity recorded on the spacecraft. This is to be related to the Jupiter stereo angle $\theta = 16.4^\circ$ (that is, the Earth-Jupiter-spacecraft angle), at this date.

Fig. 3 Projection on the ecliptic plane of the positions of the spacecraft, the Earth and Jupiter on 23 March (dashes) and 15 May (solid line). The radiation pattern of the spacecraft is indicated.



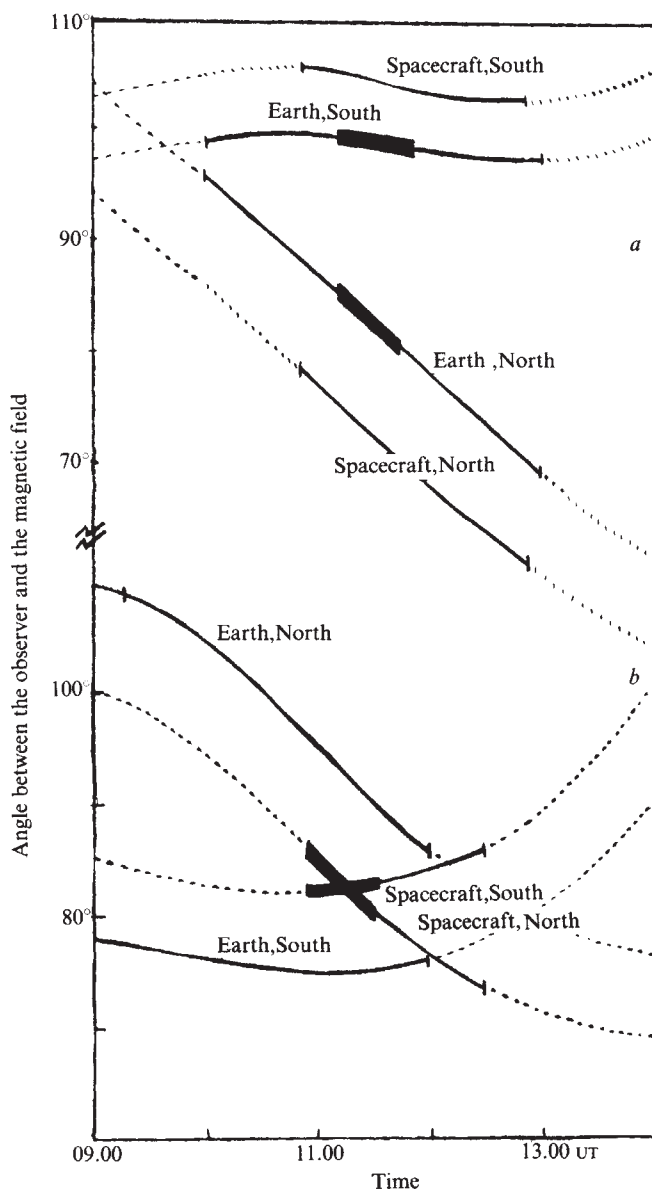
On 23 March 1974, Nançay recorded a strong Jupiter event, lasting 40 min, which reached about $3 \times 10^{-21} \text{ W m}^{-2} \text{ Hz}^{-1}$. It was visible on all the receiver outputs and showed rapid spectral structures. Aboard the spacecraft, whose sensitivity threshold was $\sim 10^{-21} \text{ W m}^{-2} \text{ Hz}^{-1}$, that is, one-third of the activity peak on the ground, no emission was recorded in the whole observation period. The stereo angle was $\theta = 14.8^\circ$.

Thus, both observations are the first direct evidence of the strong beaming of the decametric jovian emission: the recorded intensities, at the same instant, in two directions making an angle of 15° or 16° in longitude (and 0.2° or 0.4° in latitude) as viewed from Jupiter, differ by 5 or 10 dB at least.

Figure 2 shows in *L*- Φ coordinates the lines corresponding to the observation and emission periods, for the Earth and the spacecraft for the two days. The periods without emission correspond to regions of lesser occurrence probability.

In both cases, the spacecraft and the Earth swept the same ranges of longitude *L*, where the emissions occurred, whereas the ranges of Io-phase Φ were different. That seems to indicate that it is the 11° difference in Io-phase which causes the lack of correlation in the observations. Moreover, if we allow some possible variation in *L*-coordinate for the emission visibility, the

Fig. 4 Time variation of the departure angle between the observer and the magnetic field at the feet of the Io threaded magnetic flux tube; light lines, observation periods; heavy lines, emission periods. *a*, 23 March; *b*, 15 May.



Io-phase discrepancy was only 6° and 4° , respectively. Our observations may then be interpreted as an indication that the more rigorous angular condition comes from the position of Io on its orbit with regard to the observer. To illustrate this, supposing that the source of emission was at the northern or southern foot of the Io threaded flux tube (a generally accepted idea), we have computed (Fig. 4) the departure angle between the local magnetic field and the observer, using the D_4 -dipole model of Smith *et al.*⁹. On 23 March, this angle varies between 85° and 79° in the Northern Hemisphere (or 99 – 98° in the Southern) when the emission occurs on the Earth: aboard the spacecraft, during the observation period, the range was 78 – 61° (or 105 – 102°); on 15 May, the angle varies between 86° and 80° in the Northern Hemisphere (or 82 – 83° in the Southern) when the emission occurs on the spacecraft: on the Earth, the observed range was 110 – 86° (or 80 – 75°). Note that this angle differs by 9° at a given time between the Earth and the spacecraft and that, in any case, the ranges of variation overlapped. We conclude, therefore, that the decametric radiation should be emitted in a very narrow pencil beam, perhaps as narrow as a few degrees, which seems difficult to explain in terms of emission mechanism. We could imagine some strong propagation effects able to distort a less directive beam of emission.

The Stereo-5 experiment is part of a cooperative programme between the USSR (Interkosmos) and France (CNES). It was sponsored by Centre National d'Etudes Spatiales. We thank J. L. Steinberg, A. Boischot and J. W. Warwick for discussions.

M. POQUÉRUSSE

L.A. du CNRS no. 264,
Département de Recherche spatiale,
Observatoire de Meudon, France

A. LECACHEUX

Groupe de Radioastronomie décamétrique,
Département d'Astronomie solaire et planétaire,
Observatoire de Meudon, France

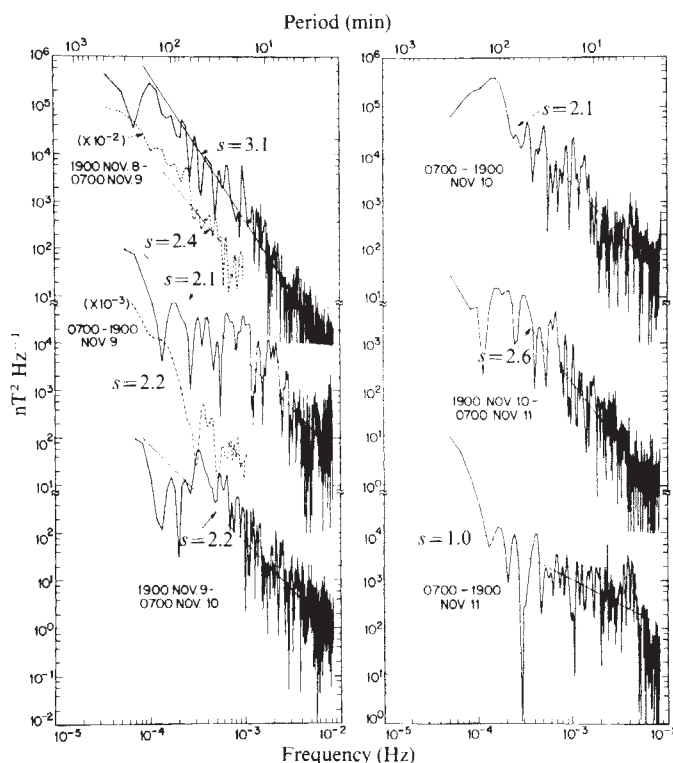
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Oscillations of the Sun and the geomagnetic field

THE suggestion by Toth¹ that the reported long-period oscillations of the solar atmosphere^{2,3} may be observed as oscillations of the geomagnetic field is an intriguing idea. It was suggested¹ that the ~ 2 h 40 min oscillations found in geomagnetic field data recorded at Tihany, Hungary, could reflect, through one of two possible mechanisms, similar period solar oscillations. In the first the geomagnetic field might have natural oscillations at this frequency that could produce environmental changes in the atmosphere^{4,5} and/or ionosphere and/or magnetosphere that would cause the ground-based telescope observations to reach erroneous conclusions. In the second mechanism the oscillations in the solar atmosphere might produce similar frequency oscillations in the interplanetary magnetic field which, through coupling with the geomagnetic field at 1 AU, would cause similar period variations in the field at the Earth's surface. Such oscillations would be in the range of periods of solar wind fluctuations measured near Earth and often attributed to Alfvén waves⁶. Regular, periodic variations in the interplanetary magnetic field with a

Fig. 1 Power spectra of six consecutive sets of 12 h of geomagnetic fluctuation data recorded at Durham, New Hampshire in 1975 (solid lines). The times and dates indicated for the spectra correspond to the local time of the station (LT $\sim$ UT $- 5$ h). The slope of the power-law fit through each spectrum is drawn. Dotted lines represent the low frequency portions of two spectra from Siple Station, Antarctica.



period near that of the reported solar oscillations have not been reported. We have spectral analysed geomagnetic field data on the same days as those published by Toth¹ using data from our fluxgate magnetometer stations in New Hampshire and in Quebec and data from the conjugate area at Siple Station, Antarctica⁷.

Our lowest latitude station (Durham, New Hampshire) is at the geomagnetic shell parameter $L \sim 3.2$; the highest latitude station (Girardville, Quebec) is at $L \sim 4.4$; Siple Station is at $L \sim 4.1$. Local time (LT) of our stations is $\sim$ UT $- 5$ h. The three components of the magnetic field are sampled at two second intervals and written digitally on magnetic tape. The instruments' noise levels are ~ 0.2 nT; the digitisation increment is 0.06 nT. We used our standard analysis method: the fast Fourier transform with a Thomson data window⁸ applied in the time domain. The geomagnetic observatory used by Toth in his analysis is located at $L \sim 1.9$, approximately 6 h east of the longitude of our stations.

Several spectra (12-h intervals) from Durham for 9–11 November 1975, the days reported by Toth¹, are shown in Fig. 1. Also shown (dotted lines) are the low frequency portions of Siple spectra for two of the time intervals. A power law frequency dependence $P(f) \propto f^{-s}$ was fit through each spectrum. The fits are shown superimposed on the spectra in the figures and the values of s for each spectrum are indicated. In none of these spectra do we find any evidence for a significant peak at a period of ~ 2 h 40 min. Because we find no significant spectral peaks in either the Northern Hemisphere data or in the conjugate (Siple) data, we can rule out a global magnetospheric phenomenon, driven either internally or externally, as the cause of the reported magnetic variations¹.

The data analysed here are expressed in power per unit frequency. At 10^{-4} Hz, the power level 10^5 nT² Hz⁻¹ corresponds to an amplitude of ~ 4 nT in a bandwidth of 2×10^{-4} Hz, an amplitude sensitivity comparable to the amplitude spectrum of Toth¹.

A relationship between the reported long period variations of the solar atmosphere and the geomagnetic field would have important implications for interplanetary field/geomagnetic field coupling, as well as for the solar wind flow in the inner heliosphere. However, we find no evidence of solar oscillations being reflected in the Earth's magnetic field on a global scale. The reported geomagnetic variations with a period of ~ 2 h 40 min must be local, related to geophysical sources in the vicinity of the observing station. We note a recent report⁹ that the envelope of modulation of geomagnetic pulsations in the period range 10–45 s as well as the auroral electrojet index AE had periods of ~ 2 h 40 min, possibly related to the solar oscillations.

L. J. LANZEROTTI
C. G. MACLENNAN

Bell Laboratories,
Murray Hill, New Jersey 07974

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Large baryon densities attainable in high energy nuclear collisions

NUCLEAR densities at least four and as much as 10 times normal should be achieved in U–U collisions with centre-of-mass kinetic energies between 1 and 4 GeV per nucleon, which would be much higher than previous discussions have indicated. Such collisions would give the best opportunity of making a stable or long-lived phase of high baryon density. It is suggested here that the accelerators at CERN would be well suited to explore the energy range of interest, both with fixed targets and with colliding beams.

Nuclear physics has traditionally focused on the properties of nuclear matter in or near 'normal' conditions, meaning those prevailing in known nuclei. The properties of systems with very high baryon density are unknown, although there have been interesting speculations about stable or nearly stable high density phases^{1–6}. In the absence of spontaneous transitions to such phases¹ (which might well be inhibited by enormous barriers) the only known way to produce high baryon density is to bombard heavy nuclear targets with heavy nuclear projectiles⁷.

Early theoretical discussions of such reactions were restricted to the nonrelativistic domain, with centre of mass (c.m.) kinetic energies much less than rest energies of the colliding nuclei. Density enhancements were computed by treating the nuclei as colliding drops of 'nuclear fluid', forming a shock front where they meet. These assumptions are valid for c.m. energies less than about 0.25 GeV per nucleon, at which the shock 'front' becomes as thick as the nuclei themselves, and a density less than four times normal is found for a plausible nuclear equation of state⁸.

The limitations on the shock wave approach suggested that higher energies could not generate still higher compressions. However, recent computations with a two-fluid model, in which bits of fluid at high relative velocity are coupled only by a frictional drag, indicated that high density could be attained during mutual interpenetration and slowing of target and projectile without shock formation⁹. An attempt to understand these results of complex numerical calculations has led to the following simple considerations, which assume minimum

compressibility and therefore give lower limits on the density enhancements to be expected at relativistic c.m. energies.

Uranium is a sufficiently large nucleus so that, at a 10–30% level of accuracy, a nearly head-on collision (≈ 0.1 b out of ≈ 7 b total reaction cross section) may be described as the meeting of two slabs 15 fm thick with nucleon density about 0.15 fm^{-3} . Of course, in the c.m. frame each slab would be Lorentz contracted, with density increased and thickness decreased by the Lorentz factor $\gamma = W/2M$, where W is the total c.m. energy and M is the mass of a uranium nucleus. Suppose that for a given γ each slab is thick enough to stop (in the c.m. frame) a nucleon in the other slab. Then the combined system will eventually be confined to a thickness less than $15/\gamma$ fm, because the back of either slab (assumed to have relativistic velocity) must progress at least halfway to the meeting plane before even a light signal from that plane could reach it. Consequently, at one moment the average baryon density will be at least 2γ times normal.

To get the maximum γ for stopping, note that a 40 mb nucleon–nucleon cross section corresponds to an average of nine interactions on crossing the slab. This number would be increased if repulsive correlations among the target nucleons were taken into account, but would be lower near the edges of the actual finite spheroidal nuclei. Suppose that the interaction probability is unchanged by previous collisions exciting the nucleon. A single high-energy nucleon–nucleon collision leaves a nearly uniform distribution in the fraction, x , of projectile momentum retained by a fast final nucleon¹⁰. If successive collisions had the same property, as they would for widely spaced target nucleons, then after N collisions the mean value of x would be $(N+1)^{-1}$, so that, converting to the c.m. frame, the stopping γ would be $1/2\bar{x} = (N+1)/2$, and the density enhancement would be greater than $N+1 = 10$ in our example. Barring an unexpected collective interaction between projectile and target, this estimate gives the upper limit on the stopping γ . A safe lower limit is $\gamma = 2$: at this γ , the target is contracted by a factor 7 in the projectile frame, giving a geometrical thickness just over 2 fm, so that in all relevant frames of observation there would be at least three collisions, each separated from the others by a time interval >1 fm (taking $C \equiv 1$). This should be long enough for collisions to be independent, so that the effective N is at least 3, giving a stopping γ of 2 and making this a self-consistent lower limit. An indication of the most likely γ should come from analysing spectra of fast nucleons emitted in high energy nucleon–nucleus collisions. Such an experiment is underway at CERN (B. Povh, personal communication).

Farley¹¹ stated that the c.m. energy range 1–10 GeV per nucleon is important. As shown here, 1–10 GeV (rather than values around 0.25 GeV suggested by older work⁸) is the range in which one would expect to see a transition from stopping to transparency, or passage of the two nuclei through each other with some excitation and deceleration. If stopping were still found at 10 GeV per nucleon, that would be a remarkable discovery of a new collective effect.

From these arguments, CERN is a logical site for exploration of the high-baryon-density frontier. Uranium ions injected into the PS could be used in all three major installations at CERN (PS, SPS, ISR) for fixed target or colliding beam experiments in the c.m. energy range 1–10 GeV per nucleon. From the technical point of view, because low energy (MeV per nucleon) partly stripped uranium beams have been made at GSI in Darmstadt, the main new development would be acceleration to energies of a few hundred MeV per nucleon and full stripping of the ions. Studies for the Berkeley Bevalac indicate that this could be accomplished by substantially improving the vacuum of the booster which feeds the PS, with little if any modification in the PS itself (H. A. Grunde, personal communication). (A plan to generate 1 GeV per nucleon uranium at the Bevalac is expected to cost \$5 million, so that an effort on a similar scale at CERN would have a capital cost roughly 1% of the annual budget¹².)

Many questions remain about observation, interpretation and possible application of phenomena associated with high baryon

density. The central point is that an almost model-independent argument shows high density can be produced, and from previous experience, explorers of such a new domain may find rich rewards—perhaps even stable dense matter.

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ALFRED S. GOLDBERGER*

School of Mathematical and Physical Sciences,
University of Sussex,
Brighton, UK

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* Permanent address: Institute for Theoretical Physics, State University of New York, Stony Brook, New York 11794.

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Photoelectrochemical reduction of aqueous carbon dioxide on p-type gallium phosphide in liquid junction solar cells

THE non-biological reduction of carbon dioxide to organic compounds is of interest, as an alternative to natural photosynthesis, for the production of organic raw materials or fuel. In one approach, the required energy was supplied by irradiation with UV light, in the presence of ferrous salts, and resulted in the production of formic acid and of formaldehyde¹. In another approach, the energy was supplied from an external power source by electrochemical reduction of aqueous carbon dioxide. The reduction of carbon dioxide and production of formic acid during the electrolysis of sodium bicarbonate in aqueous solutions has also been reported², and a study of the reduction of carbon dioxide on a mercury cathode reviews earlier work³. Polarographic measurements on mercury electrodes showed that carbon dioxide, rather than the bicarbonate ion, is the electroactive species, with a half-wave reduction potential of -2.1 V (relative to SCE), and that formic acid is the only product⁴. We report here the photoassisted electrolytic reduction of aqueous carbon dioxide, achieved using p-type gallium phosphide as a photocathode, with part or all of the energy being supplied by light. The products were formic acid, formaldehyde and methanol.

Semiconducting solids have been intensively studied for their interaction with electrolytic solutions, and the theory of interaction of illuminated semiconductors with electrolytes has been reviewed⁵. In photoelectrochemical cells, the semiconductor electrode immersed in the electrolyte is connected through an external circuit to a counterelectrode. In p-type semiconductors in the dark, there are many holes in the valence band, but only a small number of electrons in the conduction band. Illumination by light more energetic than the bandgap increases the number of electrons in the conduction band. This increase in electron density in the conduction band results in a cathodic current⁷. Electron transfer to a suitable acceptor in the solution reduces this acceptor.

The photoassisted electrolysis of water to hydrogen and oxygen has been carried out using either p- or n-type semiconductors under illumination, with metal counterelectrodes as anodes or cathodes, respectively⁷⁻¹⁰. In these cases, an external electrical bias potential was needed to drive the reaction. The use of combinations of n- and p-type semiconductors such as n-TiO₂ and p-GaP, and the illumination of both electrodes, resulted in 'self-driven' photoelectrolysis cells not requiring an external bias¹¹.

The photoelectrochemical reaction system used in the present work consisted of a closed double-walled borosilicate glass beaker (about 30 ml capacity), with thermostat set at 25 °C, containing an aqueous buffer solution as electrolyte, into which dipped a photoelectrode consisting of a single crystal of p-gallium phosphide (Zn doped; resistivity 0.19 Ω cm; front surface area 0.24 cm²; etched for 1 min in concentrated HNO₃—concentrated HF—H₂O, 3:1:4 v/v; the ohmic contact to the back surface of the crystal was made by an indium–zinc solder. The back surface and the external lead were insulated using an epoxy cement). The counterelectrode was a carbon rod (6 mm diameter), and a saturated calomel electrode served as a reference. Carbon was chosen as the counterelectrode, as it was reported that formic acid and other C, H, O compounds were not oxidised on carbon electrodes¹². In some experiments, p-GaAs and p-Si were tested as photocathodes, but n-TiO₂ and n-Si were used as photoanodes. A light-beam from a stabilised high-pressure mercury arc or from a projector halogen lamp was focused with the help of a condensing lens on to the GaP crystal. Oxygen-free carbon dioxide (purified by passing through an aqueous solution of vanadous chloride) was continuously bubbled through the electrolyte. A side-port fitted with a rubber septum and syringe served to withdraw samples without opening the flask.

Using the above cell combination, both in the dark and under illumination, a measurable current through the external circuit could be obtained only by applying a cathodic bias to the p-GaP electrode (Fig. 1a). With the application of a cathodic bias of -1.0 V (relative to SCE) to the p-GaP, the initial photocurrent density was 6 mA cm⁻², whereas the dark current density was only 0.1 mA cm⁻². The photocurrent decreased gradually, but after 24 h stabilised at about 1 mA cm⁻².

Analysis of the electrolyte solution showed the presence of formic acid (analysed by reduction with magnesium and chromotropic acid assay¹³, as well as by alkaline permanganate titration¹⁴), formaldehyde (chromotropic acid assay), and methanol (gas chromatography on Poropak Q). After 18 and 90 h of irradiation, the concentrations of formic acid produced were 1.2×10^{-2} and 5×10^{-2} M, those of formaldehyde 3.2×10^{-4} and 2.8×10^{-4} M, and those of methanol 1.1×10^{-4} and 8.1×10^{-4} M, respectively. Thus, in contrast to the reduction of carbon dioxide on metal cathodes, the photoelectrolysis on p-gallium phosphide does not stop with the production of formic acid, but proceeds further, yielding formaldehyde and methanol. The approximate constancy in concentration of formaldehyde may indicate that this product reaches a steady state of formation and further reaction.

The efficiency of conversion of radiant energy into the chemical energy (optical conversion efficiency) of the reduction products of carbon dioxide was calculated by adaptation of the formula used previously^{9,10} for the photoelectrolysis of water to hydrogen and oxygen (in which each product is assumed to be formed exclusively):

$$\text{Optical conversion efficiency} = 100 I_c [(\Delta H/Z) - V_B] / I_a$$

where I_c = current density (mA cm⁻²); I_a = incident light intensity (mW cm⁻²); ΔH = heat of combustion ($= 2.962$, 2.639 , 5.915 and 7.259 eV for hydrogen, formic acid, formaldehyde and methanol, respectively); Z = number of electrodes required in the reduction of one molecule of carbon dioxide to a molecule of product ($= 2$, 2 , 4 and 6 for production of hydrogen, formic acid, formaldehyde and methanol, respectively); V_B = electrical bias (V).

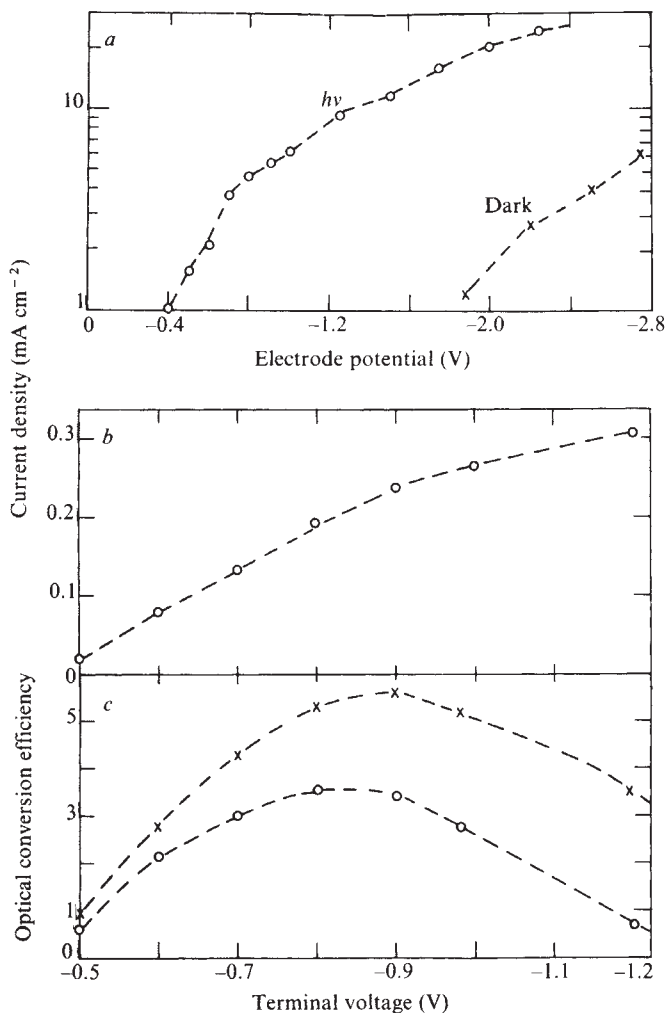


Fig. 1 *a*, Current density (log scale) as a function of p-GaP potential (relative to SCE) for the cell carbon/K₂HPO₄-KH₂PO₄, 0.05 M, pH 6.8, CO₂/p-GaP. Illumination, high-pressure Hg-lamp, unfiltered; 210 mW cm⁻². *b*, Current density as a function of terminal voltage for the cell carbon/K₂HPO₄-KH₂PO₄, 0.05 M, pH 6.8, CO₂/p-GaP. Illumination: halogen lamp, filtered to transmit 365 nm band; *I*_a 2.4 mW cm⁻². *c*, Optical conversion efficiency (%) as a function of terminal voltage for production of CH₂O (x) and CH₃OH (o) in the experiment shown in (*b*).

In an experiment using filtered light of 365 nm wavelength (Fig. 1*b*), the maximal optical conversion efficiencies for production of either formaldehyde or methanol were thus determined to occur at bias voltages of -0.8 to -0.9 V (Fig. 1*c*), and were 5.6 or 3.6%, respectively. As only 17.3% of solar irradiance (at AM2) is in the near UV and blue regions (315–510 nm)¹⁵, and thus effective for exciting p-gallium phosphide (bandgap 2.25 eV), the maximal solar energy conversion efficiency to formaldehyde or methanol is calculated to be 0.97 or 0.61%, respectively.

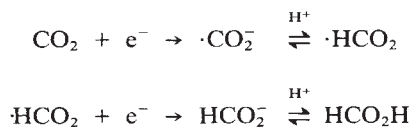
In another experiment, an n-type polycrystalline TiO₂ semiconductor was used instead of carbon as the counterelectrode, and as electrolyte a 0.1 M solution of lithium carbonate (30 ml). After 16 h of illumination (unfiltered light of Hg-lamp) on both electrodes, maintaining a constant current of 0.50 mA (current density 2.1 mA cm⁻² on the GaP cathode), and letting the negative bias on the p-GaP cathode rise gradually from -0.86 to -1.4 V (relative to SCE), the solution reached a concentration of 1.0 × 10⁻³ M methanol (or 3 × 10⁻⁵ mol). Considering that six electrons are required for the reduction of 1 mol of carbon dioxide to methanol, this corresponds to a current yield of 60%.

Using a commercial silicon solar cell (2 × 1 cm² area) as an electrode, connecting its p-junction through an external lead to

the p-GaP crystal, immersing both electrodes in 0.1 M Li₂CO₃, and illuminating the n-junction of the silicon as well as the p-GaP, no external electrical bias was required, as the two photoelectrodes produced a sufficient potential to drive the reaction. After 38 h of irradiation in the presence of carbon dioxide, the initial current of 0.05 mA had gradually decreased to 0.01 mA. During this period, the concentrations of formaldehyde and methanol reached 2 × 10⁻³ and 3 × 10⁻⁴ M, respectively.

Photoassisted reduction of aqueous carbon dioxide was also achieved using a p-type gallium arsenide single crystal electrode. However, in this case the cathodic bias required was such that no net gain in energy conversion was obtained.

The mechanism of cathodic reduction of carbon dioxide presumably involves stepwise electron transfer reactions. The first stable product, formic acid, must be due to a two-electron transfer. Possibly, the mechanism is similar to that proposed for the reduction of carbon dioxide on mercury cathodes¹⁶,



In contrast to the electrochemical reduction of carbon dioxide on metal cathodes, which stops essentially at this stage because of the high overpotential for formic acid reduction, in the case of the photoelectrochemical reaction on p-type semiconductors, the further reduction of formic acid occurs readily, producing formaldehyde and methanol.

Further development of the application of semiconducting solids to the photoelectrochemical reduction of carbon dioxide may provide a new approach to the production of organic raw materials and fuel.

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M. HALMANN

Isotope Department,
Weizmann Institute of Science,
Rehovot, Israel

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Characterisation of organic acids trapped in coals

THE characterisation of trapped organic compounds which occur in geological materials such as coal and oil shale can provide an insight into both the origin of the organic matter and its subsequent modification through chemical reactions. Much work has been done on the isolation and identification of polar and nonpolar compounds in carbonaceous materials^{1–7};

however, aromatic acids in coals have received very little attention. Here we detail the characterisation and distribution of aromatic acids extracted from lignite and bituminous coals.

Freshly ground raw lignite from Sheridan, Wyoming was first treated with 5% aqueous HCl at room temperature to free the acids from the minerals¹, and then extracted with 2.5% aqueous sodium hydroxide at 35 °C for 16 h. After work up by the usual method¹, soluble organic acids equal to 2.6% by weight of the lignite were obtained. In the same conditions, only 0.23% of acidic compounds were recovered from an Illinois no. 2 bituminous coal. No organic acids were isolated from a Pennsylvania anthracite coal. The elemental composition and description of these coals have been given elsewhere^{7,8}.

A fraction of the organic acids isolated from the lignite was derivatised with diazomethane for analysis. Another fraction was derivatised with d₆-dimethylsulphate⁹ to yield tri-deuteriomethyl-labelled derivatives. The derivatives were analysed by gas chromatography-mass spectrometry (GCMS), solid probe MS and high resolution MS using techniques which have been described previously^{7,10}. A gas chromatogram of the mixture derivatised with diazomethane is shown in Fig. 1 with numbered peaks identified in Table 1. The distribution of the organic acids as methyl esters was determined by measuring areas of GC peaks with a correction for the effective carbon number for each compound (Table 2).

Note that, as shown in Table 2, the alkaline extractable acids are qualitatively similar to those obtained from selective oxidation of the macromolecular material of the same lignite coal with aq-Na₂Cr₂O₇ (refs 7, 8), H₂O₂-CH₃COOH (refs 7, 8, 11) or 15%-HNO₃ (ref. 8).

Of particular interest is the identification of large amounts of benzene di- and tri-carboxylic acids and their derivatives which have not been found in the mild alkaline extracts of shale^{12,13}, lignin¹⁴⁻¹⁷, peat¹⁸ or humic acid^{14-17,19}. No C₆-C₃ carboxylic acids (benzene ring with C₃ side chain)^{15,20} were isolated from coals, whereas these acids are commonly found in the extracts of the above. Note also that the phenolic acids identified have only one hydroxy group but no methoxy group. This was determined from GCMS and HRMS of the sample derivatised with d₆-dimethylsulphate. This contrasts with the presence of di-, polyhydroxy, or methoxy benzene derivatives in the alkaline extracts or degradation products of the geological materials described above. The alkaline extract of bituminous coal was found to consist mainly of benzene di-, tri- and tetra-carboxylic acids (31.7% of the extract) and their methyl derivatives (39.9%), and naphthalene mono- and di-carboxylic acids (9.8%). Phenolic acids (2.2%), methylfuran di-carboxylic acid (2.9%) and aliphatic dibasic acids (4.5%) were also identified in small amounts. The remaining 9% has not yet been identified. No C₆-C₃ carboxylic acids or benzoic acid were detected. Benzene and naphthalene carboxylic acids have been identified as major

Table 1 Organic acids from lignite coal identified as their methyl esters by GC-MS and high resolution MS

Peak no.	Compound
1	Succinic acid
2	Glutaric acid
3	Methylfuranmonocarboxylic acid
4	Mixture of 3-methyl, 2,3-dimethyl and 3,3'-6,6'-tetramethyl dibasic acids
5	Benzoic acid
6	Methylbenzoic acid
7	Dimethylbenzoic acid
8	Methoxybenzoic acid (<i>m</i> - or <i>p</i> -)
9	<i>o</i> -Methoxybenzoic acid
10	Methoxymethylbenzoic acid
11	<i>o</i> -Methoxymethylbenzoic acid
12	1,2-Benzenedicarboxylic acid
13	1,4-Benzenedicarboxylic acid
14	1,3-Benzenedicarboxylic acid
15	Dimethoxydimethylbenzofuran (T)
16	Dimethylfurandicarboxylic acid
17	<i>o</i> -methylbenzenedicarboxylic acid
18	Methylbenzenedicarboxylic acid
19	Dimethylbenzenedicarboxylic acid
20	<i>o</i> -Methoxybenzenedicarboxylic acid
21	Methoxybenzenedicarboxylic acid
22	Methoxymethylbenzenedicarboxylic acid
23	1,2,4-Benzenetricarboxylic acid
24	1,2,3-Benzenetricarboxylic acid
25	1,3,5-Benzenetricarboxylic acid
26	Methylbenzenetricarboxylic acid
27	Methoxyphenyldihydrobenzofuran (T)
28	Mixture of compounds including methoxyphenyldihydrobenzofuran (T)
29	Dimethylbenzenetricarboxylic acid
30	<i>o</i> -Methoxybenzenetricarboxylic acid
31	Methoxybenzenetricarboxylic acid
32	Homologue of dehydroabietic acid (see text, T)
33	Methoxybenzyldihydrobenzofuran (T)
34	Methoxymethylphenyldihydrobenzofuran (T)
35	Dehydroabietic acid
36	Methoxymethyldibenzofuranmonocarboxylic acid (T)

T means that identification is tentative.

products from the selective aq-Na₂Cr₂O₇ oxidation of the same coal^{7,8,21}. These facts may elucidate the reactions which occurred during the transformation of organic material from biopolymers (lignin, humic acid and so on) to geopolymers (coals). It is generally agreed^{1,15-17,22} that the essential reactions, including degradation of side chains, dehydrogenation, hydroxylation, demethylation, demethoxylation, cleavage of ring structures and polymerisation, occur during these transformations. Welte has suggested²² that at the stage of coal formation (early

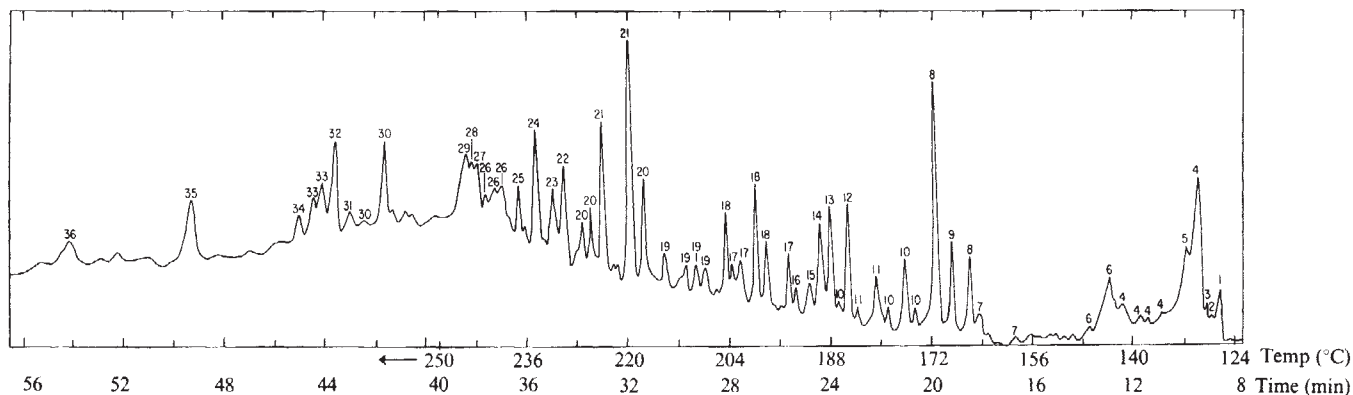


Fig. 1 Gas chromatogram of the methyl esters of the carboxylic acid fraction from lignite coal. The analysis was carried out on a Perkin-Elmer 3920B gas chromatograph interfaced to a modified Bendix model 12 time-of-flight mass spectrometer with a continually variable split between a flame ionisation detector and the source of the mass spectrometer. The separation was made on a 15.2 m × 0.51 mm SCOT column coated with OV-17 and temperature programmed over 100–250 °C at 4 °C min⁻¹.

Table 2 Organic acids identified in the alkaline extract and oxidation products of lignite coal

Acid groups	Alkaline extract (%)	Oxidation product (%)		
		aq-Na ₂ Cr ₂ O ₇ *	H ₂ O ₂ -CH ₃ COOH†	15% HNO ₃ ‡
Benzenecarboxylic acid	16.6	59.2	36.1	63.3
Methylbenzene carboxylic acid	16.0	4.3	7.6	13.9
Phenolic acid§	40.4	7.4	22.1	1.5
Furancarboxylic acid	0.8	—	15.9	3.2
Aliphatic dibasic acid	10.8	**	18.2	12.9
Terpenoid acid	6.6	—	—	—
Others	4.3	26.6¶	—	3.2
Unidentified	4.5	2.5	—	2.0

* 100% of coal was oxidised. 92% of total oxidation product is shown. The remaining 8% is humic acid-like material and nonacidic compounds.

† 80% of coal was oxidised. 50% of product is shown. The remaining 50% is humic acid-like material.

‡ 74% of coal was oxidised. 48% of product is shown. The remaining (52%) is humic acid-like material and nonacidic compounds.

§ Aromatic rings containing phenolic OH of lignite coal would have been destroyed by these oxidants except H₂O₂-CH₃COOH. The phenolic acid identified in these two oxidation products may be derived from aromatic rings containing methoxy group.

|| See peak nos 15, 27, 33 and 34 in Table 1.

¶ Carboxylic acids of naphthalene, phthalan, chroman, dibenzo-*p*-dioxin, xanthone, dibenzofuran and pyridine were identified (see refs 7 and 8).

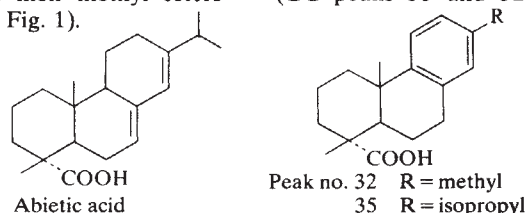
** Several acids were identified but not estimated.

—, not found.

katagenesis), functional groups are lost and CO₂ and water are produced in coals, and hydrocarbons are formed simultaneously. The benzene mono-carboxylic acid and its derivatives identified in the extracts are thought to be largely degradation residues from biopolymers at early stages of coalification, whereas the benzene di- and poly-carboxylic acids and their derivatives and naphthalene-carboxylic acids are produced in later stages by oxidative degradation. Perhaps, in the geological environment, oxidative cleavage of C-C bonds proceeded along the periphery of the coal macromolecules with rupture of bridging linkages between aromatic units. The presence of aliphatic dibasic acids in the extracts suggests that they are derived, in part, from these linkages. Indeed, oxidation of lignite and bituminous coals with various oxidants^{7,8,21,23} gave benzene di- and poly-carboxylic acids as the most abundant products; however, little or no benzene mono-carboxylic acid was found in the products.

An alkaline extract of shale was found to yield alkylbenzoic acids and phenylalkanoic acids which resemble their cyclic terpenoid precursors^{12,13}. On the other hand, no ethyl or longer-chain alkylaromatic acids, or phenylalkanoic acids were found in the present work which apparently indicates that coal has undergone more rigorous geological changes than shale. Therefore, the aromatic acids may have undergone dealkylation leaving more stable derivatives.

We have found two diterpenoid acids in the lignite extract, dehydroabietic acid and its homologue, that are apparently derived from the diterpenoid resin acid, abietic acid, which is commonly present in the resins of conifers¹⁻³. They were identified as their methyl esters^{24,25} (GC peaks 35 and 32 respectively, Fig. 1).



These two terpenoid acids are the probable precursors for related diterpenoid hydrocarbons which we have characterised in the benzene-methanol extract of this lignite⁷.

Note the presence of several furan, benzofuran and dibenzofuran acids extracted from the lignite with 2.5% aq-NaOH. These compounds may have been derived from lignans¹⁷ such as furoguaiacin and pinosresinol which are commonly found in heartwood, or as suggested by Flaig¹⁵, they might be degradation products from dibenzofuran derivatives which were formed from condensation reactions of phenolic compounds during the coalification.

In the transformation from lignite coal to bituminous and anthracite coals, the present work and our previous work on the trapped hydrocarbons in coals⁷ indicate that the following reactions probably occurred. (1) Extensive degradation of phenols; very few phenolic acids (2.2%) were found in the alkaline extract of bituminous coal, whereas the alkaline extract of lignite coal yielded 40.4% phenolic acids. (2) Loss of carboxyl group; the bituminous coal extract contained much less acids. No acids were found in the extract of anthracite coal. (3) Dehydrogenation and partial dealkylation of side chains; demonstrated by the increase in abundance of methyl aromatic hydrocarbons and a decrease in the amount of terpenoid type hydrocarbons in the organic solvent extract of bituminous coal as compared to lignite⁷.

Thus, the macromolecules in bituminous coal contain a greater proportion of aromatic structure and greater degree of crosslinking than is found in lignite. Benzene tetra-carboxylic acids and naphthalene-carboxylic acids were found in the extract of bituminous coal.

The organic acids extracted probably are both oxidative degradation products and residuals of the original coalification process. These acids, in particular the aromatic acids, were found to be characteristic of coals and different from those acids derived from biopolymers.

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RYOICHI HAYATSU
RANDALL E. WINANS
ROBERT G. SCOTT
LEON P. MOORE
MARTIN H. STUDIER

Chemistry Division,
Argonne National Laboratory,
Argonne, Illinois 60439

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Synthesis and structure of synthetic zeolite ZSM-11

ZSM-5 (refs 1, 2) is a member of a new class of shape selective catalysts which have unique channel structures with 10-membered ring openings. These catalysts have unusual properties. They can selectively convert methanol to high quality gasoline³⁻⁶ and are used in commercially significant processes, such as distillate dewaxing, ethylbenzene synthesis, xylene isomerisation and toluene disproportionation^{5,7}. The framework of ZSM-5 (ref. 2) contains a novel configuration of linked tetrahedra consisting of eight five-membered rings. These configurational units may be linked through edges to form chains and the chains to form a three-dimensional framework which describes an intersecting channel system with openings

Fig. 1 Scanning-electron micrograph of a ZSM-11 crystal. Scale bar, 1 μm .

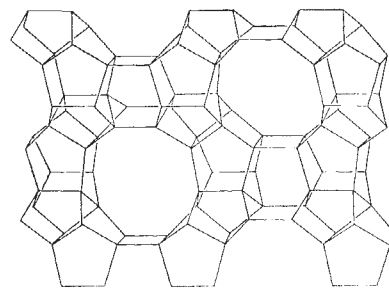
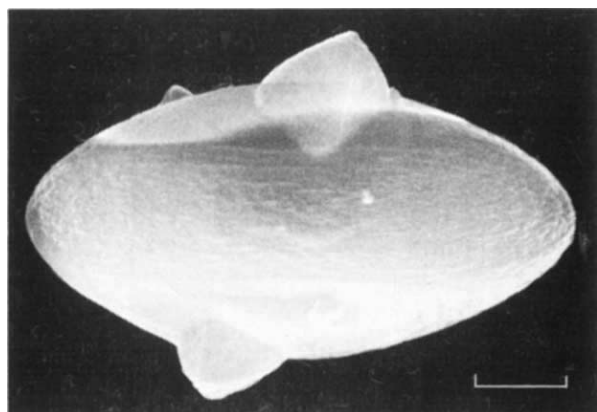


Fig. 2 Skeletal diagram of the A and B faces of the ZSM-11 unit cell. The *c*-axis is vertical. Oxygen atoms are not shown. The nearly circular 10-membered ring apertures shown are the entrances to straight channels which run parallel with the unit cell *a* and *b* axes.

intermediate between those in zeolite Linde type A and faujasite. We report here the synthesis and structure of another member of this family, ZSM-11.

ZSM-11 was hydrothermally synthesised in a system consisting of a quaternary compound, sodium oxide, an oxide of aluminum and oxide of silicon and water⁸. The single crystals of ZSM-11 have the characteristic morphology seen in Fig. 1. In the synthesised form, ZSM-11 has a typical empirical composition $[xR_4X + (1-x)M_{2/n}O]:W_2O_3:>10YO_2:ZH_2O$, where M is an alkali metal cation, W is aluminum or gallium, Y is silicon or germanium, R_4X is a cation of a quaternary compound of an element of group 5A, and $0 < x \leq 1$. Typical quaternary compounds used in the synthesis are tetrabutyl phosphonium chloride, benzyltriphenyl phosphonium chloride and tetrabutyl ammonium iodide.

The unit cell contents of the sodium form are $Na_nAl_nSi_{96-n}O_{192} \cdot \sim 16H_2O$, where n is < 16 . The resulting framework density is $17.6Si + Al \text{ k}\text{\AA}^{-3}$, compared with 17.9 for ZSM-5.

ZSM-11 crystallises in the tetragonal system with lattice constants $a = b = 20.12$ and $c = 13.44 \text{ \AA}$ which corresponds to

Fig. 3. X-ray diffraction patterns: *a*, computed from parameters in Table 1; *b*, of calcined ZSM-11.

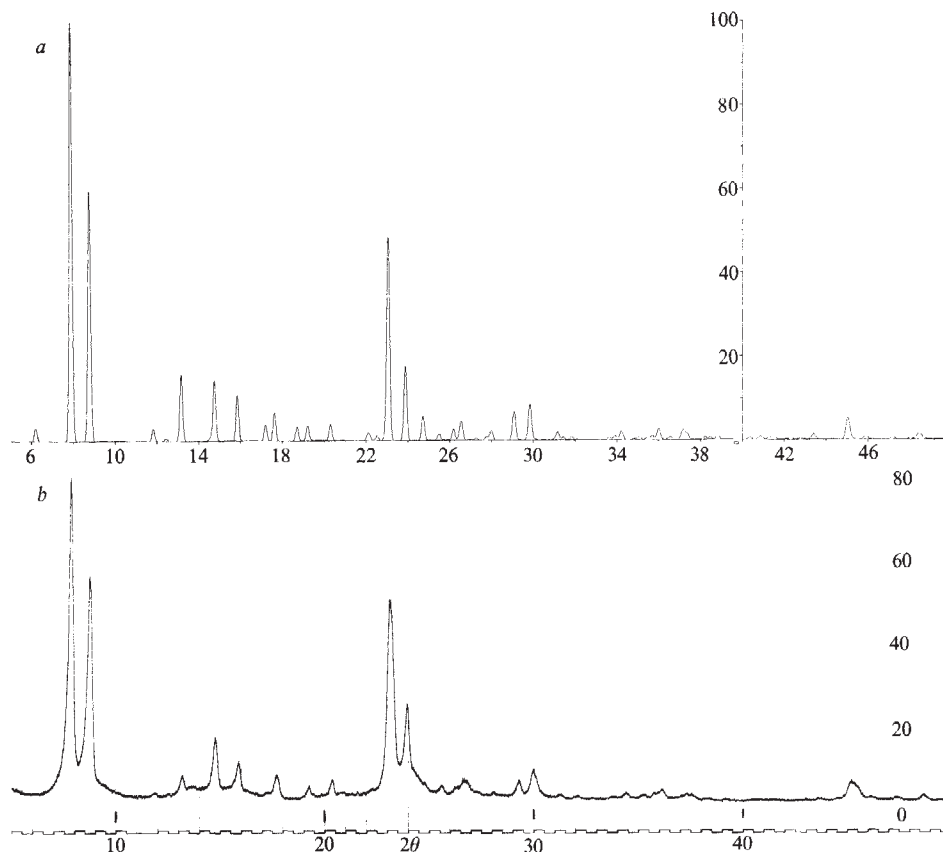


Table 1 Positional parameters for ZSM-11 framework

Atom	Position	x	y	z
T1	8g	0.0774	0.0774	0.0
T2	16j	0.1263	0.1930	0.1263
T3	16j	0.0784	0.2245	0.3477
T4	16j	0.2833	0.1969	0.1321
T5	16j	0.3118	0.0742	0.0089
T6	8g	0.1796	0.1796	0.5000
T7	16j	0.0770	0.3811	0.3610
O1	8i	0.0924	0.0	0.0208
O2	16j	0.0989	0.1207	0.0954
O3	16j	0.1115	0.2063	0.2422
O4	16j	0.2051	0.1965	0.1065
O5	16j	0.3157	0.1273	0.0981
O6	8i	0.3068	0.0	0.0533
O7	8h	0.2933	0.2067	0.2500
O8	16j	0.1126	0.1810	0.4342
O9	16j	0.2431	0.1814	0.4271
O10	16j	0.0895	0.3023	0.3707
O11	8i	0.0	0.2087	0.3441
O12	8i	0.0	0.3970	0.3836
O13	16j	0.0892	0.2480	0.0596
O14	16j	0.1230	0.4198	0.4400
O15	8h	0.0951	0.4049	0.2500

average values derived from a least-squares refinement of X-ray powder diffraction data. The systematic extinctions $h + k + l = 2n + 1$ for hkl reflections and the merging of doublets into singlets provided two major clues that ZSM-11 was a species distinct from ZSM-5 and a body-centred tetragonal variation of orthorhombic ZSM-5 with space group $D_{2d}^9 - I4m2$. Accordingly, a framework model was assembled using the chains in ZSM-5 (ref. 2), which satisfied these symmetry requirements. This was accomplished by connecting the chains together as mirror images of one another in both x and y directions (Fig. 2). In contrast, in ZSM-5 only one set of chains, in the bc plane, are mirror images of one another. The atomic positions of the silicon and oxygen atoms in this model were refined by least-squares techniques using the computer program DLS.⁹ This refinement adjusted the raw positional coordinates until the Si-O, O-O, and Si-Si interatomic distances fit the prescribed distances 1.61, 2.63 and 3.20 Å, respectively. The final positional parameters for this idealised model are given in Table 1. Comparison of the simulated powder pattern of the framework model with the X-ray powder diffraction pattern of the hydrogen form of ZSM-11 (Fig. 3) indicates that the framework model is that of ZSM-11.

In ZSM-5, linkage of chains occurs through 4-, 5-, and 6-membered rings, whereas in ZSM-11 this linkage occurs only through 4- and 6-membered rings. Nevertheless, the frequent

occurrence of 5-membered rings throughout the structure is believed to account for the high thermal stability of ZSM-11.

As in ZSM-5, the ZSM-11 framework contains two intersecting channel systems with 10-membered ring openings, but unlike those in ZSM-5 they are both straight and have the same elliptical opening with free diameter intermediate to those in Linde type A and faujasite (Fig. 4).

G. T. KOKOTAILO
P. CHU
S. L. LAWTON

*Mobil Research and Development Corporation,
Research Department,
Paulsboro, New Jersey 08066*

W. M. MEIER
*Institut für Kristallographie und Petrographie der ETH,
Zurich, Switzerland*

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A preferred orientation of intrusion of Precambrian dykes

AN analysis of the original orientation of Precambrian dykes has produced a nonrandom pattern that implies the existence of a Precambrian stress field of global extent. In general, dyke intrusion tends to occur in a north-south orientation, and is concentrated in low latitudes. This distribution has many similarities to the present distribution of sheeted diabase complexes of present day oceanic ridge crests, and may reflect the persistence of the same stress field throughout geological time. Other evidence in favour of the existence of this stress field can be found in tectonic motions or geological features that exhibit a symmetrical distribution relative to the Earth's rotational axis. For example, Moore¹ showed that the preferred orientation of spreading axes is directed north-south with a secondary maximum orientated east-west. Active transform faults exhibit a unimodal east-west distribution. A former study of recent continental rift valleys has revealed a preferred north-south orientation of fracturing. Cainozoic grabens, most of which have not been significantly rotated by plate movements following their formation, exhibit an especially strong peak within 10° of north². Here we describe our analysis of preferred fracture orientations using the trends and directions of Precambrian dykes. A central point of this study is the assumption that regional dyke patterns record fractures which result from regional stress fields operative at the time of dyke intrusion. Escher *et al.*³ have confirmed the above assumption using geological evidence from the Amitsoq dykes of Greenland.

On observing that dyke strike and magnetic declination were often closely related, Strangway⁴, proposed that the planar shape of dykes affected their acquired remanent magnetisation direction. Thus it was suggested that dykes do not record the true field direction at the time of their intrusion. This theory can no longer be sustained for two reasons. First, positive contact tests show that the magnetisation of country rocks baked by the dyke intrusion retain the same remanence direction as the dyke. Second, palaeomagnetic poles derived from dyke rocks agree

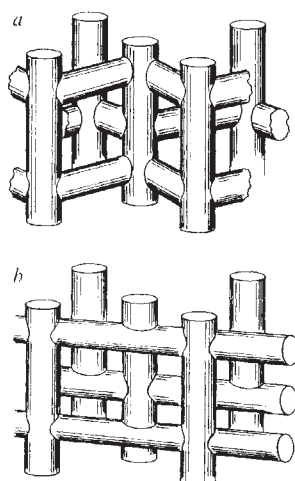


Fig. 4 Comparison of the channel structure in a, ZSM-5 and b, ZSM-11.

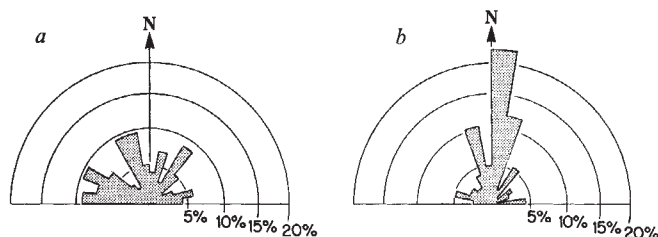


Fig. 1 Rose diagrams to show: *a*, the present distribution of the strike of Precambrian dykes; and *b*, the distribution of dyke strikes after correction to their original orientation.

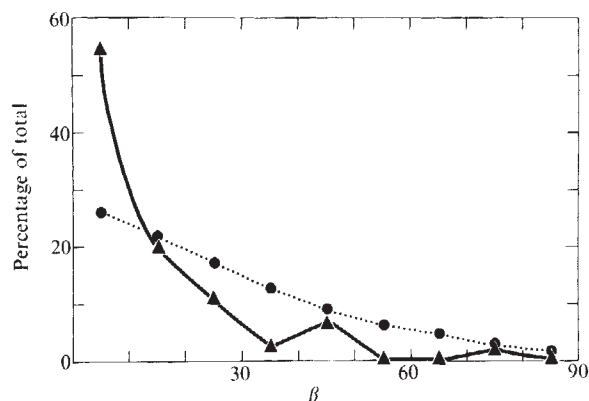
well with poles derived from adjacent contemporaneous lava flows and sills.

If Strangway's initial observation were still valid, then an alternative explanation is required for the direct relationship between original dyke strike and its remanence direction. The obvious inference is that dykes are preferentially intruded in a north-south orientation and their apparent present-day random distribution is a result of complex plate rotations subsequent to each phase of dyke intrusion. Using the palaeomagnetic remanence declination (D) and the present day dyke strike (S_p), the original dyke orientation (S_0) should simply be $S_0 = D - S_p$. World Precambrian dykes, from Archean to Hadyrian, which have been studied palaeomagnetically⁵ were combined on two rose diagrams. The data for the diagrams include dykes from both the Northern and Southern Hemispheres. As strikes are bimodal, all points are plotted in the range 270–090°. Each spot represents a swarm of dyke intrusions (such as, the Abitibi swarm, and the Sudbury dykes). Where a dyke swarm is composed of a conjugate set this is represented by two points. Where more than one study has been reported from a particular dyke suite, this is plotted as only one point.

Figure 1*a* shows the dykes in their present orientation (S_p), and Fig. 1*b* shows the distribution of the corrected dyke orientations (S_0). A simple comparison shows that a random distribution of strikes in Fig. 1*a* is brought into a clear north-south orientation in Fig. 1*b*.

Evans⁶ has noted that with increasingly higher latitudes there is an increasing statistical chance of a coincidence between original dyke strike (S_0) and remanence declination (D). Thus a random sample of dykes from subsequently higher latitudes should show a progressively increased preference for a north-south orientation. Could this explain our observed distribution? It is highly unlikely. Applying the simple relationship between magnetic inclination and palaeolatitude shows that 85% of the dykes analysed in this study have palaeolatitudes below 50°, and 40% are within 20° of the Equator.

Fig. 2 Frequency distribution of the acute angle (β) between the dyke plane and the magnetic remanence vector. The theoretical curve after Evans⁶ is for a set of vertically intruded dykes with random strike. The over-abundance of low β observations suggests the preferred north-south orientation is of geological significance. ●, Theoretical; ▲, observed.



The observed frequency distribution (Fig. 2) of the acute angle (β) (ref. 6) bears no similarity to the predicted distribution given by Evans⁶ for random orientation of dyke intrusion. As shown in Fig. 2 the expected frequency of $\beta < 10^\circ$ on a random dyke orientation model is approximately half the observed distribution. Hence it would seem that the preferred north-south mode of intrusion genuinely reflects a feature of the Precambrian global stress field. The cause of this north-south preference of dyke intrusion remains obscure; however, we note that the distribution is very similar to that now observed with sheeted diabase complexes of the ocean ridges.

Finally, as shown in Fig. 1, much of the present apparent random dyke orientation seems due to post-intrusional rotation of continental blocks. Then, where dykes from adjacent cratonic blocks can be shown to be recording contemporaneous remanences, it would be simple to determine the amount of relative rotation between the blocks since remanence acquisition (dyke intrusion). Applying this method to several dyke pairs offers the possibility of defining the sense of motions of major fault systems through geological time.

W. A. MORRIS
E. I. TANCZYK

Geomagnetic Laboratory,
Division of Geomagnetism,
Earth Physics Branch,
Ottawa, Ontario K1A 0Y3, Canada

Received 16 May; accepted 13 July 1978.

Present address: Morris Magnetics, 109–1400 Appleton Dr., Ottawa, Ontario, Canada K1B 4R9.

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Oxygen isotope palaeotemperatures from the Tertiary period in the North Sea area

THE understanding of global temperature conditions during the Tertiary period has greatly increased in the past decade owing to the successful oxygen isotope investigations of the numerous deep sea cores now available. Little attention has been paid, however, to applying the method to material from shelf sea areas, where the temperature-induced ^{18}O -record in carbonate shells are generally believed to be superimposed by ^{18}O -variations in water composition and by postdepositional alterations. This is unfortunate, as oxygen isotope data from coastal areas provide the best way of comparing traditional palaeoclimatological methods with the oxygen isotope procedure. The present study, which deals with the oxygen isotope composition of shell material from the Tertiary North Sea area, was initiated to overcome this lack of data. Here the oxygen isotope results are interpreted as palaeotemperatures and the first isotopic palaeotemperature curve for the Tertiary period in NW Europe is given.

The only reliable method of absolute palaeotemperature determinations is based on the temperature dependence of oxygen isotope exchange processes between water and shell carbonate¹. Most oxygen isotope palaeotemperature studies published recently have used foraminifera collected from the deep sea cores^{2,3}. Using foraminifera for palaeotemperature studies is difficult, however, in shelf sea areas. First the number of planktonic foraminifera is greatly reduced owing to the more restricted character of such seas⁴. Moreover, exchange processes affecting the original isotopic composition of the thin-walled foraminiferal tests are far more probable in diagenetic environments open to meteoric water than in deep sea samples.

The present study has been restricted to shell carbonate from molluscs. They are abundant as fossils in the area, and their size and often primary aragonitic mineralogy allow post-depositional alterations to be easily identified⁵. Moreover, they are known to secrete shell carbonate in isotopic equilibrium with the surrounding water⁶.

Oxygen isotope data have been obtained from samples of individual shells or combined shell assemblages collected in Tertiary strata in southern England, Holland, Germany, Denmark and southern Sweden. The material was dated by reference to the stratigraphic scheme summarised by Berggren⁷.

The isotopic results presented in Fig. 1 disclose a well defined pattern of variations in the ^{18}O -composition of shell carbonate during the Tertiary period. This pattern, however, may reflect variations in oxygen isotope composition of the Tertiary North Sea water as well as in temperatures⁸. According to Shackleton and Kennett³, the accumulation of glacial ice on Antarctica has changed the average ^{18}O -composition of ocean water by as much as 1‰ since middle Miocene time. Moreover, local deviations in oxygen isotope composition of seawater due to evaporation and fresh water mixing processes have to be taken into account. These processes vary in parallel to salinity^{9,10} and, consequently, palaeotemperature calculations have to be preceded by estimates of the salinity of the water in which the organisms grew their shells. This imposes a very severe limitation on the oxygen isotope palaeotemperature method, as no reliable procedure of palaeosalinity determination has yet been developed.

The present study has been restricted to material from faunal assemblages, the composition of which indicates normal marine environments at a certain distance from the coast. Minor fluctuations in salinity, however, are not detectable in this way. The average salinity in the Tertiary North Sea is assumed here to have varied within limits of 33–37‰, corresponding to variations in oxygen isotope composition of $\sim 1\%$ (ref. 10). The corresponding maximal error in isotopic temperatures is $\sim 4^\circ\text{C}$.

The palaeotemperature curve shown on Fig. 2 reflects these limitations. It is important to emphasise the fact that most of the ^{18}O -data have been obtained from benthonic organisms and therefore reflect bottom temperatures rather than surface conditions. However, when compared to modern conditions the investigated assemblages in most cases represent soft-bottom communities of moderate depth (30–150 m) (ref. 11). At such a depth no well defined thermocline is thought to have developed and hence—as seen in the present day North Sea—bottom temperatures are supposed to be good approximations to mean annual surface temperatures.

The isotopic palaeotemperature curve (Fig. 2) illustrates water temperatures in the southern part of the Tertiary North Sea. Temperature minima are seen in Palaeocene time (NP 4–6), middle Oligocene time (NP 22–23) and late Miocene time (NN 10), while warmer conditions prevailed in Pliocene, early and middle Miocene and specifically in Eocene time. Minor fluctuations on the curve probably reflect variations in the isotopic composition of the North Sea water.

Several studies dealing with the changing character of fossil floral and faunal communities have revealed that long-term climatic fluctuations occurred in NW Europe during the Tertiary period^{12–14}. According to these studies subtropical climates in Palaeocene time and warm subtropical to tropical conditions in Eocene time were followed by a gradual decline in temperatures, leading to cold temperate conditions at the end of the Pliocene period. Compared to these data, the temperature variations seen in Fig. 2 indicate a more drastic pattern than hitherto described.

The present curve shows discrepancies between palaeotemperature and palaeoclimatological data specifically in Oligocene time. Water temperatures are seen to have declined rapidly in the North Sea about 36–38 Myr ago. This decline may reflect the onset of cold-water circulation in the Norwegian–Greenlandic Sea initiated by the opening of the strait between Greenland and Svalbard at anomaly 13 time (37–38 Myr ago)¹⁵. However,

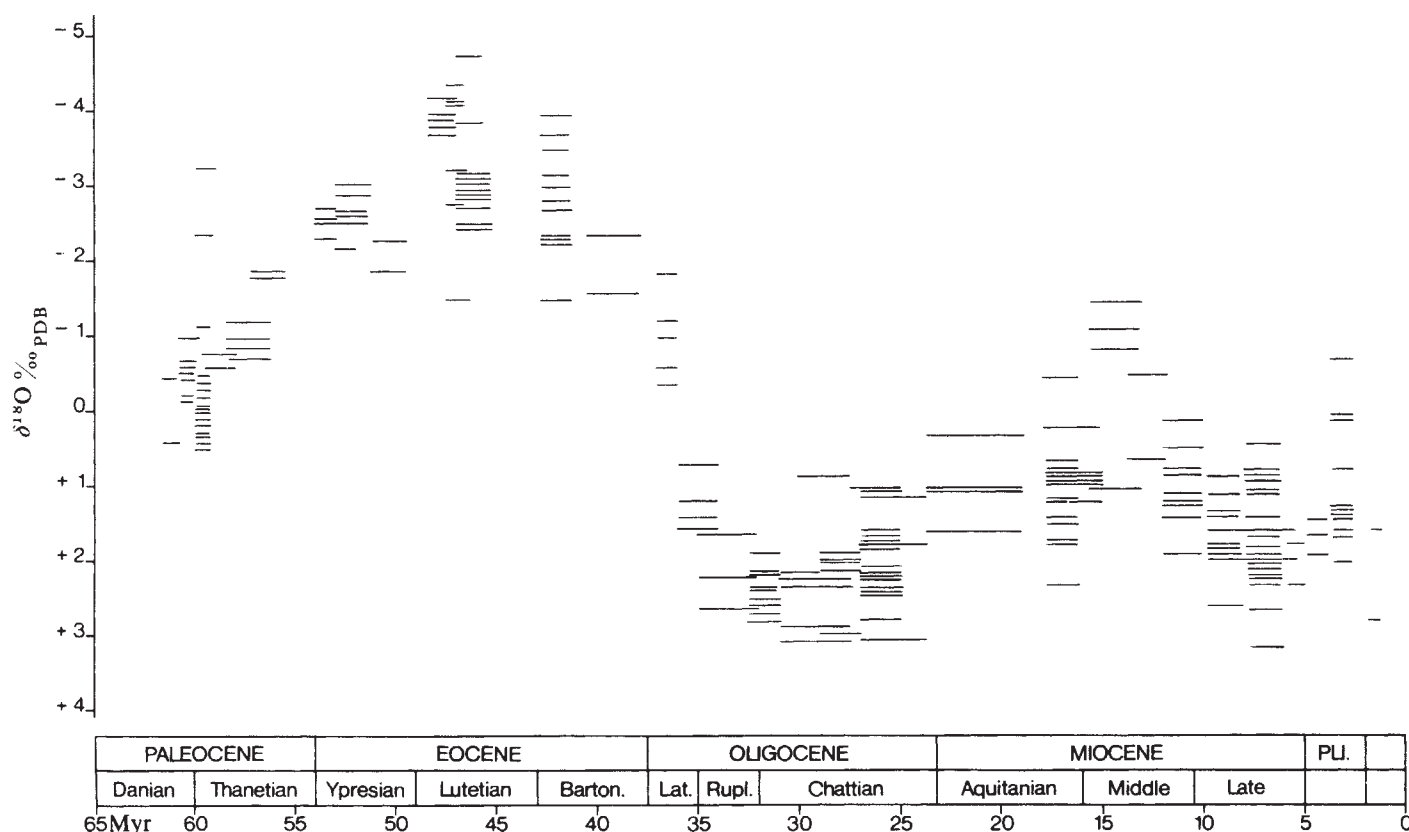


Fig. 1 The oxygen isotope composition of calcium carbonate from molluscan shells collected in Tertiary outcrops and borings in NW Europe. The horizontal bars illustrate the uncertainty in dating the analysed material. Oxygen isotope determinations have been carried out by the usual analytical procedures, material has been roasted *in vacuo* and treated with 100% phosphoric acid at 25°C . Analytical precision better than $\pm 0.1\%$.

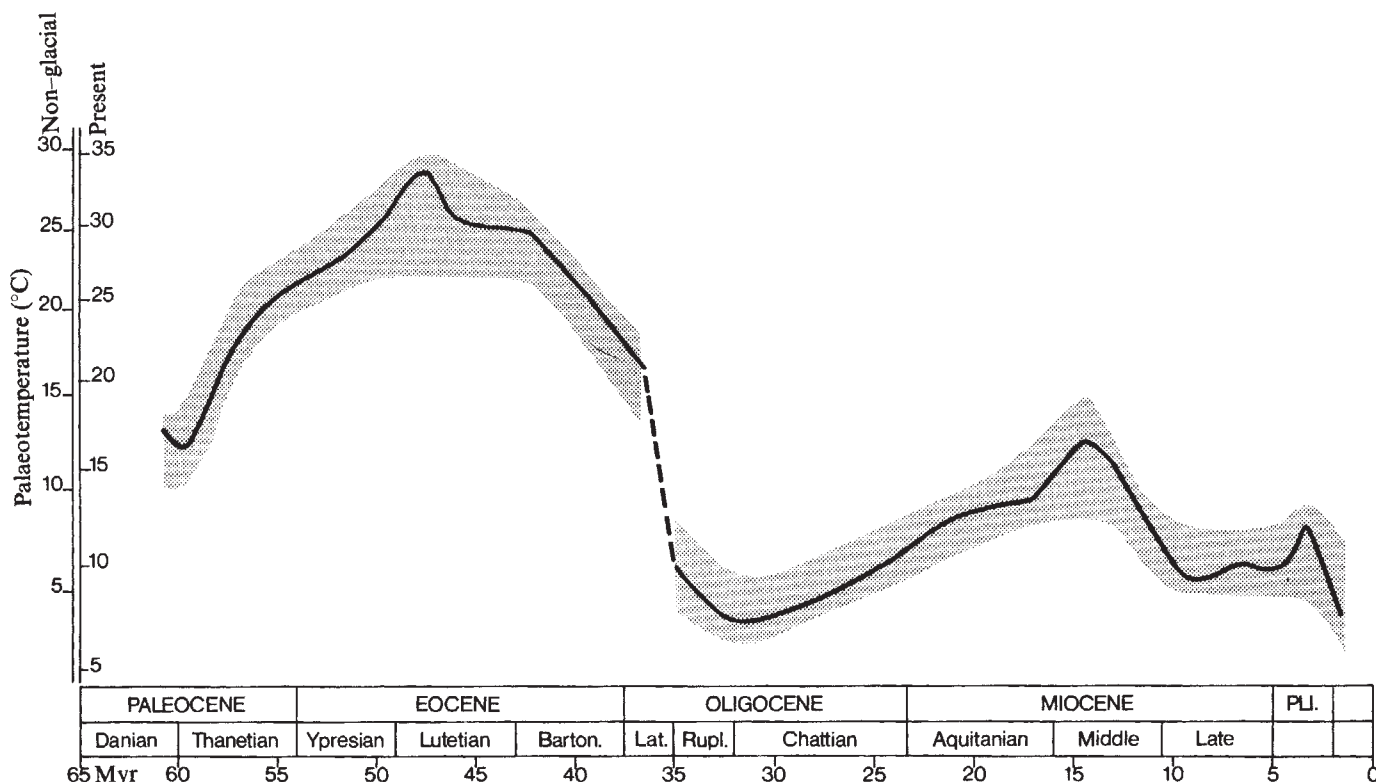


Fig. 2 Isotopic temperatures calculated from average oxygen isotope values for each locality investigated. Shaded area represents limits of uncertainty due to local variations in the oxygen isotope composition of the Tertiary North Sea water. True Tertiary water temperatures are believed to be within the shaded area. The difference between the non-glacial and the present temperature scales is caused by the shift in average oxygen isotope composition of ocean water, owing to the accumulation of glacial ice on Antarctica. According to Shackleton and Kennett³ the Antarctic icecap reached present day magnitude early in the middle Miocene.

convincing evidence from other parts of the world indicates a drastic global cooling event at the beginning of the Oligocene period, probably triggered by the development of the cryosphere¹⁶⁻¹⁸. If the unresolved problem of the stratigraphic position of the Latdorfian substage (Upper Eocene or Lower Oligocene) in NW Europe were taken into account, the isotopic shift observed between shell material from Latdorfian and Rupelian deposits may prove to be contemporaneous with the global cooling event. Thus, the low water temperatures in the Oligocene North Sea may be interpreted as being in phase with global climatic changes.

Further investigations should concentrate on the comparison between palaeoclimatological data obtained by traditional methods and the oxygen isotope curve, specifically for the Oligocene period where the extensive climatic modifications are expected to have influenced faunal and floral compositions much more than previously acknowledged. Moreover, the precise identification in NW Europe of the Oligocene global cooling event could prove to be a valuable chronostratigraphic tool in the solution of the Latdorfian Problem.

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BJØRN BUCHARDT

*Institute for Historical
Geology and Palaeontology,
University of Copenhagen,
Østervoldgade 10
DK-1350 Copenhagen K,
Denmark*

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Test size variation in *Globigerina bulloides* in response to Quaternary palaeoceanographic changes

SEVERAL different methods have been used to delineate Quaternary palaeoclimatic trends in deep-sea sediments using phenotypic variation in planktonic foraminifera including changes in coiling direction¹, pore concentration², and test surface ultrastructure³. Several workers have observed that test sizes in planktonic foraminifera may differ between different water masses⁴⁻¹⁰ but little attempt has been made to use size variations as palaeoclimatic indices within the Quaternary. The exception is based on size variations in the spherical test of *Orbulina universa* in late Quaternary deep-sea cores from the southern Indian Ocean¹¹. Oscillations in mean diameter of this species closely match late Quaternary palaeoclimatic changes: larger mean diameters occurring during warm episodes. We

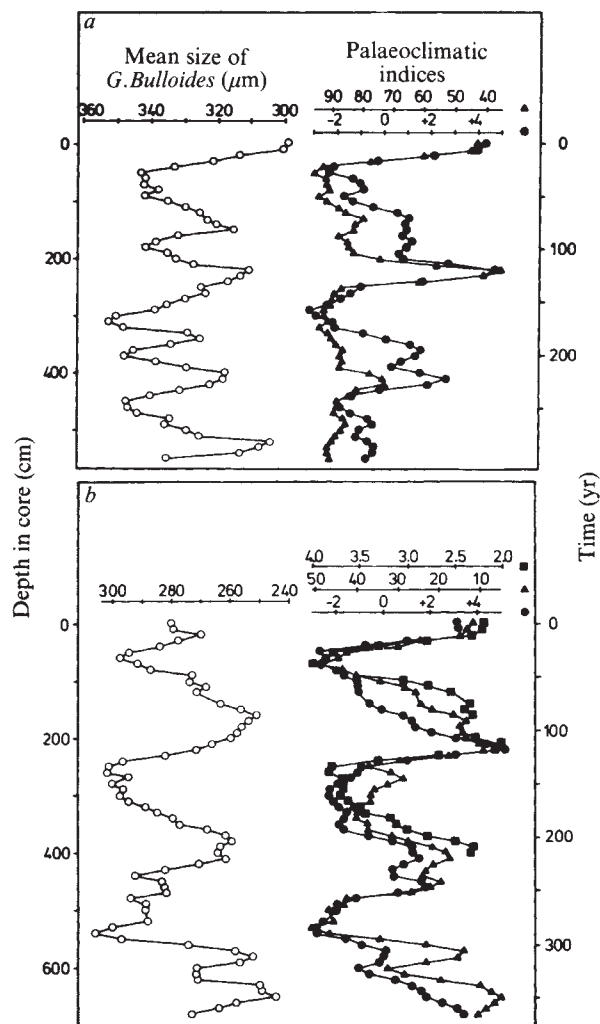


Fig. 1 Variation in mean sizes of *G. bulloides* in relation to three palaeoclimatic indices in cores *Eltanin* 49-19(a) and 48-22(b) from the southern Indian Ocean. The curves are plotted as three-point moving averages. 'Warm' is to the right and 'cool' to the left. Oxygen isotopes and other data for E48-22 are from Williams¹⁴. Chronology plotted on right is based on extrapolation of ages (assuming constant sedimentation rates) from Termination II (128,000 years ago¹⁵) which is at 235 cm in E49-19 and 236 cm in E48-22. ●, Palaeoclimatic principal component; ▲, % sinistral *N. Pachyderma*; ■, oxygen isotope ratios (δ¹⁸O)

report here that changes in mean sizes of *Globigerina bulloides* can also be used as a palaeoclimatic index in the late Quaternary.

We have examined mean size oscillations of *G. bulloides* in three late Quaternary cores from sub-Antarctic waters of the southern Indian Ocean¹² (Table 1). The results from two of the cores are presented here. The third core examined (E45-74) shows conspicuous evidence of calcium carbonate dissolution, and the data revealed no meaningful relationships between size parameters and climatic history. Previously⁸ we have shown that in surface sediments of the southern Indian Ocean, *G. bulloides* increases in mean size with decreasing surface-water temperature, and we wished to test if this trend could be usefully applied to down-core palaeoclimatic studies. The mean size oscillations were compared in both cores with palaeoclimatic curves established from principal component analyses of abundance data of planktonic foraminifera¹³ and frequency changes in coiling proportions of *Neogloboquadrina pachyderma*, and in E48-22 also with a curve based on ¹⁸O/¹⁶O ratios.

In core E49-19 from the central sub-Antarctic, the mean size oscillations of *G. bulloides* are very similar in period and amplitude to the palaeoclimatic curves (Fig. 1) indicating a strong

Table 1 Data for *Eltanin* cores used in the study

Core	Latitude (S)	Longitude (E)	Water depth (m)	Core length (cm)	Age of sequence (kyr)
E48-22	39°53.7'	85°24.6'	3,380	680	370
E49-19	43°53.2'	90°06.0'	3,036	550	300

palaeoclimatic control of the mean size variation. A cross-correlation coefficient of 0.63 ($P < 0.1\%$) indicates that the curves are statistically significantly correlated. Mean sizes are larger during cool episodes and smaller during warm episodes; this pattern therefore agrees with the distribution in surface sediments⁸. Thus, latitudinal shifts in water masses during the late Quaternary created latitudinal displacements of isopleths of mean sizes of *G. bulloides*.

In core E48-22 from the Subtropical Convergence, there are also strong correlations among the mean size curve of *G. bulloides* and the palaeoclimatic indices (Fig. 1). However, in contrast to E49-19, the mean size curve is not exactly in phase with, but is slightly off-set from the other curves. Cross-correlation analyses indicate that it is off-set from the variations in coiling ratios of *N. pachyderma* and oxygen isotopic ratios by 10 cm (to ~5,500 yr) and from the palaeoclimatic principal component by 40 cm (to ~22,000 yr). These differences do not, however, impair the almost perfect correlation between mean sizes and climatic history.

Palaeotemperatures have been estimated for E49-19 and E48-22 using the following palaeotemperature equation established from the mean size variation in *G. bulloides* in surface sediments⁸:

$$\text{Temperature (annual-average surface-water in } ^\circ\text{C)} = -0.1184 (\text{mean width of } G. \text{ bulloides in } \mu\text{m}) + 45.06.$$

The calculations show that surface-water temperatures ranged between about $3^\circ\text{C} \pm 1^\circ\text{C}$ and $10^\circ\text{C} \pm 1^\circ\text{C}$ at the site of E49-19 (central subantarctic) and between about $9^\circ\text{C} \pm 1^\circ\text{C}$ and $16^\circ\text{C} \pm 1^\circ\text{C}$ at the site of E48-22 (Subtropical Convergence). Our estimates for E48-22 agree closely with the temperature range of 6°C to 15°C calculated previously for this core using different parameters¹⁴.

We have therefore shown that mean size of *G. bulloides* in the late Quaternary of the subantarctic is strongly related to palaeoclimatic trends, and hence this parameter represents a potentially valuable new tool for palaeoclimatic analysis in this area.

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B. A. MALMGREN

Department of Geology,
Stockholm University, Box 6801, S-113 86 Stockholm,
Sweden

J. P. KENNETT

Graduate School of Oceanography,
University of Rhode Island, Kingston,
Rhode Island 02881

Received 3 July; accepted 28 July 1978.

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Genetic differentiation during speciation

SPECIATION theory is still largely descriptive. How many and what kind of genes are implicated in speciation is a central unresolved problem of evolutionary biology^{1,2}. Does speciation require major genomic changes³⁻⁵ or may minor ones suffice^{1,6-9}? Similarly, does speciation depend on structural or on regulatory genes¹⁰? We have investigated these questions with reference to the actively speciating fossorial mole rats of the *Spalax ehrenbergi* complex in Israel, which comprises four morphologically indistinguishable chromosome forms ($2n = 52, 54, 58, 60$)¹¹ adapted in that order to increasing aridity¹². Narrow hybrid zones between karyotypes¹³ and mate selection¹⁴ (through olfaction¹⁵, vocalisation¹⁶ and aggression¹⁷) suggest that the recently¹⁸ formed species represent progressive stages of final speciation¹³. Genic diversity proved low, and genic similarity between karyotypes very high, in the previous test based on 17 gene loci of tissue proteins⁸. The test of eight additional loci of blood proteins, which is reported here, reinforces earlier conclusions and sheds light on the allozyme-environment association and on the amount of genetic differentiation during speciation.

In the previous study⁸, 383 adult mole rats comprising all four karyotypes were tested for 17 gene loci. In the study reported here blood samples of 499 specimens, collected during 1969-1971 from 21 populations of the four karyotypes, were tested for eight gene loci, six in the serum (haptoglobin, *Hp*; transferrin, *Tf*; albumin, *Alb*; slow α_2 globulin (*Gl*); esterases, two scorable loci, *Est-1* and *Est-4*) and two in the haemolysate (haemoglobin, *Hb*, and a non-haemoglobin erythrocyte, *Prot-1*). Routine vertical starch gel electrophoresis was conducted on the separated blood samples. Details of collecting sites and electrophoretic methods and results are available from us. Of the eight loci examined, six (*Hp*, *Gl*, *Alb*, *Est-1*, *Hb* and *Prot-1*) were monomorphic and fixed for the same allele in all 21 populations tested. Only two loci, *Est-4* and *Tf* were polymorphic, with three segregating alleles in each. Transferrin is the only locus, both of the blood and tissue proteins, which displays almost complete alternative fixation: $2n = 54$ is nearly fixed for *Tf^a*, whereas $2n = 52, 58$ and 60 are either fixed or nearly so for *Tf^b*; the rare allele, *Tf^c*, appears only in $2n = 60$. Mean genetic indices for the *S. ehrenbergi* complex and for $2n = 52, 54, 58$ and 60 , respectively, are: *A* (mean number of alleles per locus) = mean 1.25 (1.16, 1.28, 1.20, 1.36); *P* (mean proportion of polymorphic loci per karyotype; criterion for *P* 1%) = mean 0.20 (0.12, 0.24, 0.16, 0.28), and *H* (mean proportion of loci heterozygous per individual) = mean 0.039 (0.035, 0.016, 0.037, 0.069). Genetic identity, *I*¹⁹, between the four karyotypes, is remarkably high (mean 0.966, range, 0.931-0.988).

The results support the hypothesis that habitat specialists display significantly lower genic variation than habitat generalists²⁰. Mole rats, like most fossorial mammals⁹, are habitat specialists living apparently in a relatively predictable environment which presumably selects for homozygosity (homoselection), though exceptions exist²¹. The evidence negates genetic drift due to small population effects, inbreeding in relatively semi-isolated populations, or the time hypothesis²² as explanatory models. First, the genetic pattern is characterised by general uniformity of alleles at 24 out of 25 loci in 21 populations across the range of the four abundant karyotypes. Second, *H* is apparently not correlated with time. Evolutionary divergence time for the *S. ehrenbergi* complex, deduced from electrophoretic data²³, is within the last $250,000 \pm 20,000$ yr, in accord with the hybrid zone¹³ and fossil²⁴ evidences. Yet $2n = 60$, presumably the last derivative of speciation 75,000 yr ago, has the highest *H*, not the lowest as expected by the time divergence hypothesis²². Heterozygosity seems to correlate with spatial environmental heterogeneity. The largest range, that of $2n = 60$ (see map in ref. 11), is also the most variable

spatiotemporally in terms of ecological heterogeneity and uncertainty, extending in both Mediterranean and arid environments.

Concerning speciation theory, the *Spalax ehrenbergi* complex represents a remarkable case of speciation with relatively few genomic changes. The nature and extent of hybridisation in *S. ehrenbergi*¹³ reinforces the hypothesis that the chromosome forms are young, closely related species at final stages of speciation. A growing body of evidence from newly formed unrelated plant and animal species²⁵ suggests that the essence of speciation, that is the development of pre- and/or postmating reproductive isolation *per se* may occur with little genomic change, either genic or chromosomal. In many cases the apparent genetic distance between species is the result of post-speciation evolutionary divergence. In the fossorial rodent complexes of *Thomomys talpoides*⁹ and *S. ehrenbergi*, where all stages of evolutionary divergence from large to zero gene flow between diverging populations are known, further development of ethological isolating mechanisms based on the few chromosomal rearrangements which initiated the reproductive isolation have been accomplished without many allelic changes. This pattern is also known in other species²⁵, including some parasites whose speciation involves few changes in either structural or regulatory genes, and also rarely involves chromosome rearrangements². The hypothesis that regulatory rather than structural genes are implicated in speciation¹⁰ is yet to be tested. Until tests of regulatory genes become available it seems plausible to assume that speciation may occur with relatively little genetic change.

Species vary in kind in accord with different strategies of evolutionary adaptations and speciation patterns. Reproductive isolation may either follow or precede the genic or chromosomal divergence of gene pools²⁶, and so speciation may be associated with either major or minor alterations in structural and/or regulatory genes, as well as with chromosomal evolution. The extent of genetic differentiation depends on various correlated and interacting factors, including the taxon, mode and rate of speciation; population and migration size and structure; breeding and mating system; reproductive strategy, chromosomal rearrangements and polyploidy, and the ecological selection structures. To become both predictive and falsifiable, speciation theory must be based on critical multifactorial tests incorporating the genetic system, ecological regime and historical patterns in actively speciating populations.

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EVATAR NEVO
HARTWIG CLEVE

*Institute of Evolution,
University of Haifa,
Haifa, Israel
and*

*Institut für Anthropologie und Humangenetik
der Universität München, FRG*

Received 4 November 1977; accepted 28 June 1978.

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Limit of single vision in stereopsis depends on contour sharpness

OUR two eyes see slightly different images of the world, and this enables us to estimate the position of objects in depth. When we look at a fixed point in space we can correctly judge the distance of an object that is in front of, or behind this point by the amount of disparity of its retinal images. Our brain combines these disparate images in such a way that we can see the object as single within a certain limit of disparity. This limit is called Panum's fusional area; for fine-line targets the disparity limits are found to be ± 7 min arc for central (foveal) vision, and larger for peripheral vision (for review see ref. 1). However, Fender and Julesz² demonstrated that fusion may be extended beyond Panum's area when random-dot stereograms are used. Experiments reported here demonstrate that Panum's area also increases for stimuli with blurred contours, that is, for contours containing lower spatial frequencies.

Figure 1a shows three gratings of sinusoidal luminance profile; the adjacent gratings are in antiphase, that is, each light bar in one grating corresponds to a dark bar in the other. Each grating is surrounded by a contour and provided with short vertical lines at the top and bottom to facilitate fusion. The first

task of the observer is to fuse the top lines of the top gratings (the bottom left grating and the rest of the page should be covered). Fusion of the top lines is easier when a cardboard partition is put between the gratings, so that each eye views only one grating. It will be seen that after the short lines and the rectangular outline are fused, both the gratings can also be fused (although at first the light bars seem superimposed on dark bars and vice versa). They are seen as a single grating either closer or further away than the short lines (as observed previously by Blakemore and Hague³). The criterion of appropriate fusion is that the top short lines are seen pointing between the light and dark bars of the grating for observers with good binocular vision. (If an observer has one eye strongly dominant, this eye will determine the perceived position of the short line). This fusion is not affected by viewing and fusion of the bottom instead of the top short lines, except that now the short lines are seen in the middle of a light or dark bar of the grating. In both viewing conditions, single vision and stereopsis are easily established over a wide range of viewing distances and this finding was confirmed on 15 observers with normal binocular vision. This indicates that the disparity range of single vision is at least as large as the width of one bar (or a half-cycle of the grating), which at a viewing distance of 10 cm is about 4° .

Now cover the right grating, uncover the left bottom grating and rotate the figure by 90° so that two horizontal gratings (in antiphase) can be viewed by left and right eyes, respectively. It will be seen that now fusion becomes difficult and even if it occurs (most often by suppressing one eye) it does not produce stereopsis.

The next demonstration is concerned with the square-wave gratings (Fig. 1b) in antiphase. Such patterns, with sharply outlined contours, are known to be interpreted in the visual system by detection of their edges^{4–7}, which contain higher spatial harmonics⁸.

First try to fuse the top short lines using the same method as when viewing Fig. 1a. Now, however, a simultaneous fusion of the top short lines and the gratings is very difficult, as the sharp edges of the gratings in antiphase seem to produce rivalry, otherwise the edges are not seen as sharply. In spite of this, all 15 observers saw the square wave grating in depth, either closer or

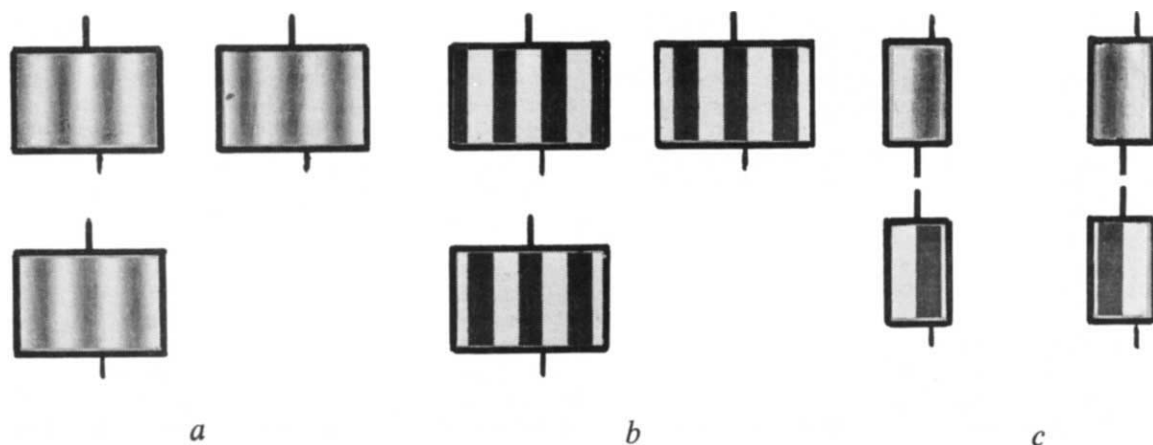


Fig. 1 Demonstration of depth perception with and without single vision of contours. *a*, Sinusoidal gratings in antiphase can easily be fused, after fusing the short line in the top two figures (all other figures should be covered by a white sheet of paper). Observers finding this task difficult should put a piece of cardboard, in the sagittal plane, between their eyes and the two gratings, so that the left and right eyes view only left and right gratings, respectively; start viewing the figure at a distance of 20–30 cm and then gradually reduce the viewing distance. Note that the vertical gratings are fused and perceived at a different depth from that of the short fixation lines over a wide range of distances (down to <10 cm) and that the gratings do not produce the depth effect if the whole display is rotated through 90° . *b*, Square-wave gratings in antiphase can be fused initially only at a distance over 30 cm, but gradually also at smaller distances. The square-wave grating is then seen in depth relative to the short lines, but the sharp edges of the grating become double or unclear at distances <15 cm. It is important that the observer sees the gratings in focus with both eyes (otherwise, defocused gratings lose their sharp luminance profile and become similar to the sine gratings). Viewing the gratings horizontally produces only binocular rivalry. *c*, Fusion of single bars of either cosinusoidal or sharp (square) profile is equally easy when looking at the gaps between the short lines in the middle of the figure: both bars are seen as single and in depth. However, the sharp edges of the bars are seen either double or unclear when the observer looks at the bottom short lines at viewing distances <10 cm.

farther away than the short lines, and 12 of them reported rivalry at the same time. Only three observers (all of whom had a strongly dominant eye) reported seeing sharp edges without any rivalry, possibly by suppressing the rival contour produced by the nondominant eye. This suggests that the nondominant eye may contribute to stereopsis without producing a rival double image.

Although the periodicity of the patterns in Fig. 1a and b helps in seeing them in depth (as several contours have similar disparity⁹), it is not a necessary condition for this phenomenon. Figure 1c shows two pairs of single bars corresponding to those in Fig. 1a and b. Now, however, for comparison the sharp bars (bottom) and the 'blurred' bars with a consinusoidal profile (top) are presented simultaneously in the same disparity with respect to the short lines. Some observers find it difficult to fuse the short lines between the bars, but after several attempts it is usually possible to perceive the bars as being closer than the short lines (if the left and right eyes look at the left and right lines, respectively). The sharp edges of the square-wave bars, however, are seen as double.

All the phenomena shown in Fig. 1 suggest that objects with sharply outlined contours and patterns with gradual luminance gradients have different ranges of disparities over which they are seen as single, or at least not seen as rival double images. In other words, the range of single vision in stereopsis is inversely related to the spatial frequencies present in the pattern, as though there were many Panum's areas instead of one determined with fine lines.

Neurophysiologists¹⁰⁻¹² have noted that units with small receptive fields, representing central vision, often have a narrower range of disparities than units with large receptive fields representing peripheral vision. However, the phenomena reported here are concerned mainly with central vision (note that the bars in Fig. 1c may be made shorter without affecting the percept). Graham, Robson and Nachmias¹³ have demonstrated that central vision has a range of mechanisms tuned to different sizes (or different spatial frequencies). Also, Maffei and Fiorentini¹⁴ noted that cortical cells subserving each part of the visual field are tuned to a range of sizes (spatial frequencies). Although specific neurophysiological investigations relating the range of disparities to the receptive field size in the same part of central vision have not yet been published, some experiments hint that such a relationship may exist. Simple cells in the cat visual cortex responsive to low spatial frequencies tolerate a greater lateral misalignment of line stimuli presented dichoptically than the cells responsive to higher spatial frequencies (D. J. Tolhurst, J. D. Thompson and C. Blakemore, personal communication).

Other psychophysical experiments also point to different binocular processing of sinusoidal and square-wave gratings in antiphase (J.J.K. and I. Wood, in preparation). The binocular contrast thresholds for the detection of sinusoidal gratings were the same whether the gratings were in-phase (identical) or in antiphase for the two eyes. However the thresholds for binocular viewing of square-wave gratings were higher when the gratings were in antiphase as compared with the thresholds for the in-phase presentation. This finding may be of clinical importance, for as the sinusoidal gratings do not produce rivalry irrespective of their phase they could be used for exercising binocular vision in patients with squint.

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J. J. KULIKOWSKI

Visual Sciences Laboratory,
Department of Ophthalmic Optics,
UMIST, PO Box 88,
Manchester, UK

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Telephoto lens system of falconiform eyes

VISUAL ACUITY in falconiform birds has been shown to be higher than man. For instance, vultures with eyes similar in size to those of man have a grating detectability of about twice the spatial frequency of man¹. This is consistent with measured image quality in an eagle eye similar in size to the human eye². Recently a falcon (*Falco sparverius*) has been shown to have a grating detectability 2.6 greater than that of man³. Although the image quality of these falconiform eyes is at least twice as good as man, their minimum intercone spacing is only slightly less than in the human retina. Unless the ratio of focal length to the axial eye length of these birds greatly exceeds that in man, falconiform eyes, unlike man, would be unable to resolve their best retinal image quality. We show here that the presence of a spherical depression in the deep fovea of falconiforms may act like the negative lens component in a telephoto lens system or opera glass. The focal length of the birds' dioptrics can then in theory exceed the axial length of the eye, thus providing the relatively large images necessary for more complete image reconstruction (high resolving power) in a localised region of the retina.

The anatomical resolving power of an eye is set by the image size and by the receptor grain. The image size is specified by the focal length, f , while the receptor grain is specified by the centre-to-centre spacing of foveal cone, d . It is convenient to define an anatomical resolving power, RP , as the reciprocal of the angular separation between cone centres:

$$RP = f/d \quad (1)$$

In order to determine precisely the intercone distance, d , we made photoreceptor counts by light microscopy on fresh material and by light and electron microscopy on fixed material in the deep foveas of numerous birds ranging from large eagles to small falcons and, for comparison, in human foveas⁴. In all situations shrinkage was carefully monitored and corrected for, and only the most central region of the fovea was examined. In all falconiforms studied $d \approx 2 \mu\text{m}$, independent of their eye size, while $d \approx 3 \mu\text{m}$ in man. In man, focal length $f \approx 17 \text{ mm}$ (ref. 5) while in falconiforms f (believed to be accurately estimated from the curvature of the retina^{6,7}) measured in all our birds was found to be $f \approx 0.65L$, where L is the axial eye length. Using equation (1), the anatomical resolving power of falconiforms with eyes the same size as man ($L \approx 24 \text{ mm}$) is only 1.38 times that of man. If the resolving power of these birds is to equal twice that of man, then f must equal 22.6 mm or 1.45 times that measured from the retinal curvature. We next show that this amount of image magnification is in fact consistent with the optical function of the bottom of the foveal pit.

Falconiforms are well known to have a deep nasal fovea^{8,9}, see for example, Fig. 1, where Fig. 1b shows the deep fovea of the red-backed hawk, *Buteo*, which also has a 'man-sized' eye. We call attention here not to the steep sides of the fovea^{8,10}, but rather to the fact that the bottommost portion of the pit is a well formed hemisphere. Our hypothesis is that this concave portion of the foveal pit (labelled by the arrow in Fig. 1b) functions as a negative lens which, together with the positive power of the cornea and lens, acts like the telephoto optical system of Fig. 2. This system has an effectively long focal length which, in

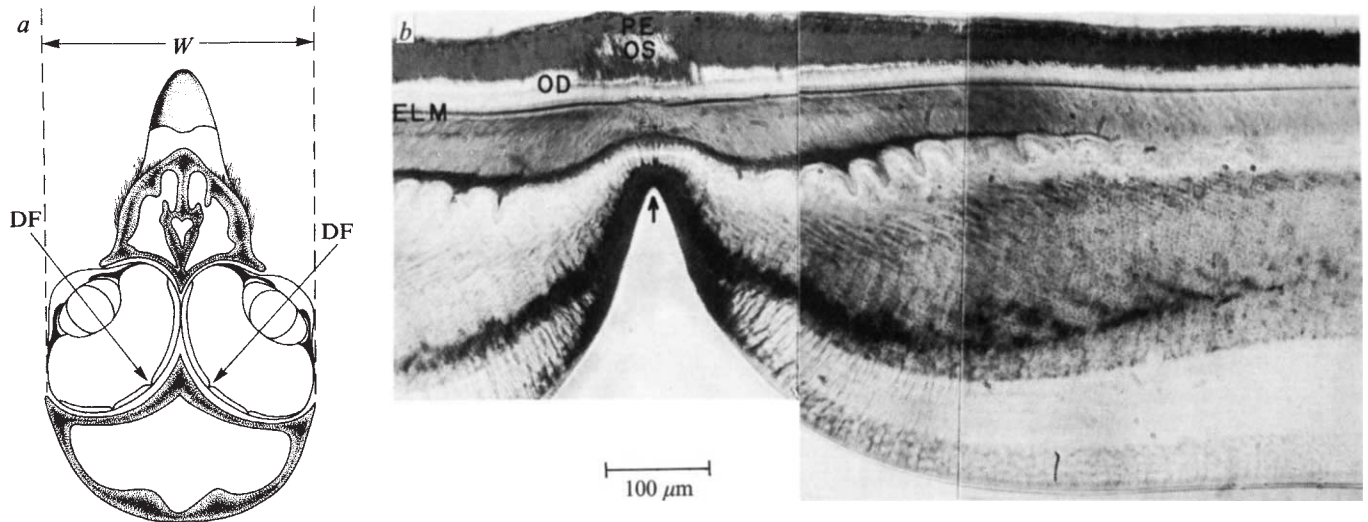


Fig. 1 *a*, Schematic of an avian eye viewed ventrally, illustrating the relative position of the deep or nasal fovea (DF). The figure is adapted from the hawk *Buteo latissimus*¹⁴. *W*, the head width, approximately equals twice the axial eye length *L*. *b*, Unstained 10- μ m thick section of glutaraldehyde-fixed retina of red-backed hawk in region of deep fovea. Section photographed using interference contrast. Increasing darkness indicates higher refractive index. Arrow indicates concave region of foveal pit which we hypothesise may function as a diverging optical element to project magnified image on receptors at centre of fovea. Note that *n* is uniform between internal and external limiting membranes outside of fovea on extreme right of figure. Dark densities sclerad to ELM are the result of pigment and do not necessarily relate to *n*. ELM, external limiting membrane, OD, oil droplets; OS, outer segments; PE, pigment epithelium. Scale bar, 100 μ m.

theory¹¹, can even exceed the physical length of the eye. Walls⁸, ignoring the bottommost portion of the pit, suggested that the steep sides of the foveal pit magnifies the image, but Pumphrey¹⁰ showed that instead they cause distortion.

Thus, from Fig. 2 the effective focal length of the bird eye is given by mf , where m is the magnification¹¹ of the foveal pit, defined in the legend of Fig. 2*b*, and f is the focal length used in equation (1). Therefore, from equation (1), the resolving power of falconiforms is mf/d . The magnification m increases the smaller the radius of curvature R of the concave pit and/or the greater the distance s of the bottom of the pit from the photoreceptors. From our above discussion, m must equal about 1.45 for the man-sized falconiform eye to have twice the resolving power of man.

Using the deep fovea of the red-backed hawk in Fig. 1*b* as an example, with $s = 110 \mu\text{m}$ and $R = 6 \mu\text{m}$, then the refractive index n_r of the deep fovea must equal 1.369 if m is to be 1.45. This result is found from the equation in the legend of Fig. 2*b* assuming the refractive index of the vitreous humor¹² is the same as man and cat⁵, that is, $n_c = 1.336$. There are several convincing reasons why n_r should equal or exceed 1.369. First, the vitreal surface of the foveal pit is formed by Müller cells only. From our electron micrographs, these Müller cells are seen to be exclusively and densely packed with microfilaments. Similar protein, for example, collagen in the cornea, has a refractive index¹² $n_r \approx 1.55$. Second, interference microscopy of the red-backed hawk fovea, (Fig. 1*b*) indicates an n_r similar to that of the cone outer segments¹³ for which $n_r \approx 1.4$. Since the regions of highest n in Fig. 1*b* are shown as the darker areas, the region below the pit has a nonuniform refractive index. Thus, Fig. 2 represents only a crude model of the optics. We emphasise that the high refractive index region surrounding the spherical pit in Fig. 1*b* is absent in extrafoveal regions and in all primate foveas and less apparent in the shallow foveas of falconiforms. Third, measurement of interference fringe displacement in the deep fovea of a glutaraldehyde fixed, Spurr's media embedded specimen (Fig. 1*b*) indicates a minimum value of 1.379 for the refractive index of both the pit and sides of the deep fovea. However, this value is a lower bound because we have not adjusted for the fact that the high n Spurr's medium infiltrates the denser tissue more than the less dense tissue. The measured n value is consistent also with the intensity of the foveal reflex

believed equal to that of myelinated nerve fibres of the optic disk¹⁴. Thus, the very high refractive index below the spherical pit and its radius of curvature are consistent with the pit functioning as a negative element in a telephoto lens system.

The deep fovea may also act as a focus indicator; however, both the nail polish and the ray models used to test this hypothesis¹⁵ were not scaled to give the correct magnification factor as determined by our interference microscopy and anatomical measurements (L. Harkness, personal communication). When appropriately scaled, we have observed the magnification predicted by the telephoto lens model and furthermore, the entire 'retinal' image is in focus in the same plane that magnification occurs.

There are distinct disadvantages associated with the telephoto lens system described above, including a narrow field of view for high resolving power and susceptibility to aberrations caused by the steep sides of the fovea. Why is it then that the avian eye did

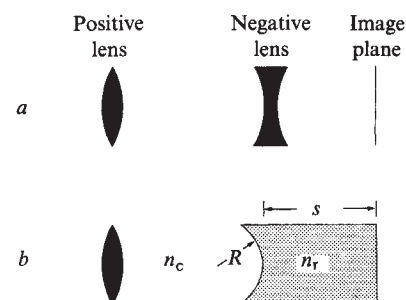


Fig. 2 Schematic of the telephoto lens. *a*, Classical design. *b*, Equivalent system of avian eye where the negative lens is replaced by a spherical (concave) surface. The magnification of the system,

$$m = 1 + \frac{s}{R} \left(\frac{n_r - n_c}{n_c} \right)$$

where n_r and n_c are the refractive indexes of the medium to the right and left of the spherical surface respectively, R the radius of curvature of the surface and s the distance from the apex of the spherical surface to the image plane. The position of the oil droplets is taken as the image plane in Fig. 1*b*.

not simply have a higher cone density, avoiding these difficulties? The answer may be found in the constraints imposed by the wave nature of light. Because of frustrated total internal reflection¹¹, photons within one outer segment have a probability of crossing over into neighbouring outer segments. This optical 'cross talk'¹⁶ increases the more densely packed the cones become, manifesting itself by an increased reduction in contrast sensitivity. If high resolving power is the animals' primary objective, the deep fovea, acting as the magnifier of a telephoto lens, may be a strategy superior to a fine receptor grain.

Although our arguments have been applied mainly to falconiforms with man-sized eyes, they should hold more generally. In all of the falconiforms we have studied, the focal length f of equation (1) is directly proportional to axial eye length L while the minimum inter-cone spacing d and magnification m due to the deep fovea are independent of eye length. Consequently the resolving power, RP , of these birds should be directly proportional to L or equivalently to the animals' head width W of Fig. 1a. Our finding that the birds' pupil diameter, as measured in bright natural conditions, is directly proportional to L is also consistent with the RP being directly proportional to L . This follows assuming, as in man¹⁷, that the birds' best image quality (minimum line spread) is nearly diffraction limited and occurs at the smaller (bright light) pupil diameters.

Finally, recall that grating detectability provides only a limited assessment of bird visual superiority. Alternatively consider a distant point object. The most crucial determinate of detection is then maximum image brightness which also equals the two-dimensional polar area under the modulation transfer function of the optics¹¹. From the modulation transfer function for the optics^{2,17}, we calculate that the man-sized eagle eye can, in theory, detect a distant point object at a distance 3 to 8 times greater than man. This ratio is nearer to 8 when the object is significantly brighter than its surround, and ~ 3 when the object brightness is equal or less than its surround. Such superior visual acuity is in better agreement with the reported observations¹⁸ than the value of twice that of man found from grating detection¹.

Thus man-sized falconiform eyes have an image quality at least twice as good as man², yet their minimum inter-cone spacing is only slightly less than man⁴. Nevertheless, our anatomical findings and refractive index measurements are consistent with the hypothesis that the spherical portion of the deep fovea acts like the negative lens component in a telephoto lens system. The focal length of the dioptrics may then be sufficiently extended for the bird to resolve its superior retinal image in a localised region.

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ALLAN W. SNYDER
WILLIAM H. MILLER

*Institute of Advanced Studies,
Departments of Applied Mathematics and Neurobiology,
Australian National University,
Canberra, Australia
and
Yale University School of Medicine,
Department of Ophthalmology and Visual Science,
New Haven, Connecticut 06510*

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Cyclophosphamide delays 3-methylcholanthrene sarcoma induction in mice

THERE is increasing evidence to show that suppressor T lymphocytes facilitate the growth of neoplasms that have tumour-specific transplantation antigens¹⁻⁶. Studies on suppressor cell activity with respect to various non-tumour antigens have shown that treatment of mice with cyclophosphamide depresses suppressor cell-mediated mechanisms to a greater extent than the effector arm of the immune response⁷⁻⁹, provided the proper dose and timing for drug administration was used. Based on these observations we speculated that treatment of mice with cyclophosphamide during the latency period of tumour induction might prevent (or at least delay) the appearance of primary tumours. We show here that this hypothesis is correct. In BALB/c mice given 3-methylcholanthrene (MCA) and injected with cyclophosphamide (2 mg per mouse) every 10 days, the appearance of primary sarcomas was delayed. Because the effects were striking, and were obtained with one of the drugs most commonly used in cancer chemotherapy, we decided to report the data. We are, however, aware that explanations for the drug effect other than interference with suppressor cell activity are equally possible, including a direct effect of the drug on tumour cells.

BALB/c mice were bred by brother to sister mating at the Fred Hutchinson Cancer Research Center. Female mice, 6-8 weeks old, were randomised into six experimental groups, each group comprising 50 mice except group 6 which had 45 mice. All mice received an intramuscular injection into each thigh of 0.1 mg MCA dissolved in triolein. The day of MCA injection is referred to as day 1 of the experiment. Two groups (1 and 4) were injected subcutaneously on 4 occasions (days -10, 20, 50, 80) with 10^6 heavily irradiated cells per mouse from tumour 1423. This tumour is an MuLV antigen negative MCA-induced BALB/c sarcoma; these tumour cells were pre-irradiated with 15,000 rad *in vitro*⁶. The tumour cells were injected to determine whether any immunisation to putative common TSTA could be obtained. Two other groups (2 and 5) were similarly injected with cells from normal BALB/c kidneys, while the remaining two groups (3 and 6) received phosphate-buffered saline (PBS) only. Cyclophosphamide was injected intraperitoneally at 10-day intervals in a dose of 2 mg per mouse (corresponding to approximately 100 mg per kg body weight at the onset of the experiment), starting 13 days before MCA and continuing throughout the experiment, in groups 4, 5 and 6. This dose of cyclophosphamide was chosen on the basis of experiments performed in an allograft system, which examined suppressor cell activity controlling the cell-mediated allograft response in BALB/c mice¹⁰. The time schedule was based on the evidence that suppressor T cells have a half life of less than 4 weeks¹¹. Groups 1, 2 and 3 were injected with PBS instead of cyclophosphamide. The previously eartagged mice were observed at weekly intervals for tumour development; the treatment

Table 1 Delayed appearance of primary MCA induced sarcomas in mice given cyclophosphamide during the latency period

Group	Cyclophosphamide* (+ or -)	Immunisation†	No. of mice with tumour at various times after MCA‡						No. of mice dead without tumour d142	Total no. tumours per group d142	% Mice without tumour d142
			d84	d97	d104	d119	d131	d142			
1	-	1423 Tumour	1	28	30	46	48	50	0	73	0
2	-	Normal kidney	4	16	24	44	46	48	1	69	2
3	-	None	6	13	20	41	42	46	4	62	0
1-3	-	1-3 combined	11	57	74	131	136	144	5	204	0.7
4	+	1423 Tumour	1	2	4	18	24	25	3	32	44
5	+	Normal kidney	0	3	3	14	24	31	1	35	36
6	+	None	1	1	2	12	18	26	0	31	41
4-6	+	4-6 Combined	2	6	9	44	66	82	4	98	40
Statistical significance of differences between groups (1-3) and (4-6) $P <$			0.02	0.001	0.001	0.001	0.001	0.001	NS	0.001	0.001

* 2 mg cyclophosphamide per mouse intraperitoneally 13 d before MCA and at 10 d intervals afterwards.

† 10^6 cells irradiated with 15,000 rad *in vitro*, were injected subcutaneously 10 d before MCA and at 30 d intervals on three more occasions.

‡ Data pooled from mice with unilateral or bilateral tumours. All groups comprised 50 mice except group 6, in which there were 45 mice. Mice were inoculated with 0.1 mg MCA into each thigh on day 1.

of the mice was unknown to the observer. The appearance and growth of tumours in each thigh was recorded. Statistical significance was calculated by χ^2 analysis.

As shown in Table 1, groups receiving cyclophosphamide developed tumours much more slowly than did untreated groups. At all times of observation except the first (84 days after MCA), the differences were statistically significant at the $P < 0.001$ level; 84 days after MCA, the P value was < 0.02 . At the time of writing, 142 days after injection of MCA, all but one of the mice in the controls had at least one tumour, whereas 40% of the mice in the cyclophosphamide treated group remained tumour free. However, the major effect was probably a delay of tumour appearance rather than a prevention of tumour induction, since the appearance of new tumours in the drug treated group has not levelled off. The cyclophosphamide treated mice have shown no signs of sickness and have increased in weight at the same rate as mice not receiving the drug. The few non-tumour related deaths observed have been no more frequent in the drug treated groups (Table 1).

There was no difference in tumour induction between groups which were immunised with tumour 1423, or injected with normal kidney or PBS. Thus, there was no evidence for any immunisation to antigens shared by the 1423 sarcoma and primary MCA sarcomas. This observation agrees with previously reported failures to immunise against MCA carcinogenesis by giving MCA tumours during the latency period¹², but does not exclude that immunisation with other tumours, either induced by MCA or in another way, may be effective¹³. The failure of 1423 sarcoma cells to immunise against any putative common TSTA was not altered by cyclophosphamide treatment (group 4).

The delayed tumour appearance in groups that received cyclophosphamide is consistent with the hypothesis that cyclophosphamide inhibits tumour growth by interfering with either a suppressor cell mechanism or an immunostimulatory mechanism of the type described by Prehn¹⁴. However, it is equally consistent with a non-immunological interpretation. Cyclophosphamide may, for example, have destroyed neoplastic cells as they developed or it may have interfered with carcinogenesis. That we do not know the mechanisms of the drug effect in this system should not, however, detract from the fact that a very substantial delay in primary tumour induction was obtained, using a protocol which has few apparent side effects. Work in progress in our laboratory, using cyclophosphamide treatment in conjunction with adoptive transfer of lymphoid cell populations containing suppressor cells, should

determine whether the drug has any effect on suppressor cells during chemical carcinogenesis.

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INGEGERD HELLSTRÖM
KARL ERIK HELLSTRÖM

Division of Immunology,
Fred Hutchinson Cancer Research Center,
Seattle, Washington 98104
Departments of Microbiology, Immunology and Pathology,
University of Washington Medical School,
Seattle, Washington 98195

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Neoplastic transformation of human diploid cells *in vitro* after chemical carcinogen treatment

ALTHOUGH many unsuccessful attempts have been made to transform normal human cells to the neoplastic state¹⁻⁴, the putative transformations reported have been primarily with cell lines^{5,6} derived from xeroderma pigmentosum biopsies or osteosarcoma tumour tissues⁷⁻⁹. Recently, cells from a skin biopsy taken from the lip of an adult female were reported to be transformed by 4-nitroquinoline oxide and *N*-methyl-*N*¹-nitronitrosoguanidine¹⁰ (MNNG). We now report that primary or low passage cells *in vitro* from various human foreskin cell populations can be neoplastically transformed *in vitro* by six

different chemical carcinogens. A close correlation between growth in soft agar and tumour formation in nude mice was obtained.

Randomly proliferating human cell populations have an extremely rapid DNA repair system that is exceedingly proficient and error free^{11,12}, as over 90% of the damage caused by the chemical carcinogen is repaired within 4–10 h (ref. 13). When normal diploid human cell populations were exposed to known chemical carcinogens, transitory abnormal cellular, morphological and culture characteristics, such as polygonal morphology, criss-cross orientation of the fibroblast cells and loss of density-dependent inhibition were expressed. We have not yet been able to regularly demonstrate transformation of randomly proliferating cell populations prepared from different skin tissue samples. However, reproducible transformation occurred when scheduled DNA synthesis was altered and proliferation of the normal (unaffected) portion of the cell population was impaired. To obtain transformation the normal cell populations were blocked in G₁/S interphase, then

treatment with PMA, insulin, anthralin or oestradiol. The range for cells in S was 67–95% with a mean value of 90% of the cells going through S phase of the cell cycle for a population of 1.5×10^6 cells in these culture conditions. Carcinogen stock solutions had been prepared by dissolving the chemical in acetone and diluting with 100 ml of CM immediately before use. The concentrations used were made by further dilutions of the stock solutions with CM. After the treatment period, the cell cultures were washed three times with CM and immediately sub-passaged 1:2 into CM containing 8× nonessential amino acids (MEM, Flow) and 2× vitamins. When the cell populations were 75% confluent, the cultures were serially passaged 1:10. This ratio of subpassaging the cultures was continued for 20 population doublings (PDL). The saturation density of the transformed cell populations examined always exceeded the density of the normal untreated cell populations (Table 1).

After 20 PDL, 50,000 cells were seeded into 2 ml of 0.35% agar or 1.0% methylcellulose (6,000 c.p.s.) supplemented with

Table 1 Population density and colony-formation capability in 0.5% agar of control and carcinogen treated cell populations

Chemical carcinogen ($\mu\text{g ml}^{-1}$)	No. of treated populations*	Cell density† ($\times 10^6$ per 75 cm^2)	No. that grow in soft agar‡	Frequency of colony formation in agar
0	—	1.7	—	—
PS (5.0)	7	4.0	7	$1:10^{3.5}$
β -PL (13.0)	4	9.0	4	$1:10^{3.7}$
Af-B ₁ (10.0)	7	4.5	7	$1:10^4$
MNNG (0.1)	8	3.0	6	$1:10^5$
4-NQO (0.002)	10	6.0	7	$1:10^6$
EMS (10.0)	7	8.0	5	$1:10^{3.5}$

The following were added at PDL 1.5: PS, β -PL, aflatoxin B₁ (Af-B₁), MNNG, ethyl methane sulphonate (EMS), 4-nitroquinoline-1-oxide (4-NQO).

* Primaries were derived from 10 different foreskin samples per treatment and cultures of each were used with chemical carcinogens.

† Mean cell population densities ($\bar{M}$ -cell density $\times 10^6$ per 75 cm^2) were determined 7 d after 1:4 split. These values represent 10 different experiments.

‡ Fifty-thousand cells were seeded into 2 ml of 0.35% agar over a 5 ml 2% base in 25 cm^2 wells. These cultures were incubated at 37°C in a 4% CO₂-enriched air atmosphere and re-fed on day 7 with 0.5 ml of medium-supplemented soft agar. The base agar medium was supplemented with RPMI 1629 and the soft agar was supplemented with Lo-Cal medium (Biolabs). Colonies, >100 cells per colony, were counted 9 d after seeding.

released and treated with the appropriate carcinogen during the S phase of the cell cycle. After carcinogen treatment proliferation of the unaffected cell population was purposely inhibited. Moreover, if morphologically altered colonies were selectively removed and grown in conditions that restricted proliferation of unaffected cells in mass culture, there was an increase in the growth of the abnormal cell populations.

Randomly proliferating cell populations obtained from 10 foreskin tissues dispersed with collagenase¹⁴ were sub-passaged in complete medium (CM) composed of minimum essential medium–25 mM HEPES (Gibco) at pH 7.2 supplemented with 10% fetal bovine serum (FBS) (Rehatuin), 1 mM sodium pyruvate, 2 mM glutamine, 0.2% sodium bicarbonate, $5.0 \mu\text{g ml}^{-1}$ gentamycin, in an atmosphere of 4.0% CO₂ enriched air at 37°C . Preconfluent (70–80%) logarithmically growing cultures were transferred to Dulbecco's modified Eagle's medium (Biolabs) lacking arginine and glutamine, at pH 7.2 supplemented with 10% dialysed FBS to decrease cell multiplication. After 24 h, the amino acid-deficient medium was replaced with CM to which either 0.5 IU of insulin or $1 \mu\text{g ml}^{-1}$ 17- β -oestradiol, anthralin, or phorbolmyristate-acetate (PMA) had been added. Ten hours later, chemical carcinogen in CM was added with either PMA, anthralin, insulin or oestradiol. This time coincided with the time when the maximum number of cells were in S phase of the cell cycle. Both chemical carcinogen and PMA, anthralin, insulin or oestradiol were removed 12 h later. This time interval coincided with the end of the S phase of the cell cycle (8.3 h). The absolute number of cells in S varied from tissue to tissue and

modified Dulbecco's Lo-Cal growth medium (Biolabs) over a 5 ml 2% base supplemented with RPMI 1629 growth medium–20% FBS. After incubation for 17 d in a 4% CO₂-enriched air environment at 37°C , colonies formed. Agar-containing cultures were re-fed every 7 d with 0.5 ml of Lo-Cal medium supplemented soft agar. The frequency of colony formation in soft agar ranged from $10^{-3.5}$ to 10^{-6} and for methylcellulose from 10^{-6} to 10^{-7} for the different carcinogen treated cell populations at PDL 20 (Table 1). These colonies were isolated from each semisolid growth medium and seeded into CM containing nonessential amino acids and grown until confluent. When 20,000 cells again were seeded into soft agar, or methylcellulose, the colony frequency approached 100%.

A comparison of the frequency of growth in soft agar, or methylcellulose, indicated that PDL 20, after treatment was discontinued, was a suitable PDL for determining the ability of transformed cell populations to grow in soft agar. Cell populations with a high capacity for growth in soft agar or methylcellulose also grew to relatively higher cell density than normal cultures (Table 1). For example, aflatoxin B₁-treated cell populations at PDL 28 grew in soft agar at $1:10^4$ or methylcellulose, at a frequency of $1:10^{5.4}$ after 17 d. At this PDL the population density in a treated flask was 4.5×10^6 cells per 75 cm^2 after 4 d, while the population density in the control 75 cm^2 flask was 1.7×10^6 cells. At PDL 40 the frequency of growth in soft agar was $1:10^3$ and in methylcellulose it was $1:10^{4.6}$.

After the colonies were removed from the semisolid medium and grown in culture these cells were reseeded into soft agar:

Table 2 Tumour growth in nude mice of same cell lines treated with chemical carcinogens

Cell population	Agar derived cell lines tested	Mice with tumours/ mice injected
PS	3	7/11
β -PL	1	3/4
Af-B ₁	3	8/14
MNNG	1	3/5
4-NQO	1	2/4
EMS	1	3/4

At PDL 40–50 the transformed cell populations previously removed from the soft agar were injected into irradiated mice at a cell density of 5×10^6 cells per nude mouse. A 'tumour take' was described as a nodule, 0.5 cm in diameter, that was palpable within 4 weeks after injection. None of the untreated cultures at cell densities of 10^7 cells produced tumours when injected into the mice.

100% of the seeded cell populations formed colonies. A similar response was observed with propane sultone (P.S.), β -propiolactone- or aflatoxin B₁ (Af-B₁)-transformed cell populations, whereas only 25%–70% of the reseeded cell populations, treated with 4-NQO, ethyl methane sulphonate, methyl methane sulphonate, or MNNG, formed colonies in soft agar.

Colonies were removed from the soft agar and serially propagated to a cell density of $10^{6.3}$. The ability of these cells to grow in soft agar was correlated with tumour formation in the nude (*nu/nu*) mouse (Table 2). Preliminary chromosome analysis confirmed that all cell lines examined had a human chromosomal constitution with a diploid or near diploid stem line. G-band analysis demonstrated that some lines, despite their normal diploid chromosome number, contained numerical deviations or abnormal chromosomes. C-band analysis revealed other alterations such as pericentric inversions and chromosomes with large areas of heterochromatin or abnormal chromosomes with more than one centromere. These transformed cells were injected into the subscapular region of the *nu/nu* mice previously irradiated with 450 rad (ref. 15). The bleb regressed between 24 and 96 h depending on the size of the inoculum. Subcutaneous tumours appeared in the subscapular areas 18–25 d after injection of the cell mass. After 4–6 weeks, the tumours (0.8–1.5 cm) were surgically removed, part was examined histopathologically and the rest transplanted into other mice. The tumours were composed of multiple vacuolated cell types predominantly a syncytial network with eosinophilic cytoplasm and ovoid basophilic nuclei. There

were also foci of more centrally very vacuolated basophilic staining cells with central or peripheral nuclei (Fig. 1). The presence of these cells composing the foci in the tumour mass is consistent with presence of proliferating exogenous cells producing the tumour mass. The tumours were classified as undifferentiated mesenchymal tumours, and the tumour incidence was recorded (Table 2). To date, all cell lines derived from agar injected into nude mice produce tumours. Tumours have been serially subpassaged $3 \times$ to date. Palpable growths formed in 4 weeks and reexamination of these growths confirmed their mesenchymal origin.

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GEORGE E. MILO, JR

Department of Physiological Chemistry
and Veterinary Pathobiology,
1900 Coffey Road,
Ohio State University,
Columbus, Ohio 43210

JOSEPH A. DIPAOLO

Biology Branch,
NCI-NIH,
Building 37,
Bethesda, Maryland 20014

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Resorbing bone is chemotactic for monocytes

MONOCYTES are often found adjacent to bone-resorbing surfaces. Their function at these sites is unknown, although they have been shown *in vitro* to have the ability to resorb bone^{1,2}. There is also strong circumstantial evidence that circulating monocytes are precursors for the major bone-resorbing cell, the osteoclast^{3–7}. The mechanism by which circulating monocytes are attracted to sites of bone remodelling are unknown. We have examined the products of resorbing bone for chemotactic effects on circulating human monocytes and found that organ cultures of resorbing fetal rat long bones produced factors which were chemotactic for human peripheral blood monocytes, but not for neutrophils or lymphocytes. The chemotactic effect of resorbing bones occurred independent of the humoral mediator of bone resorption. The mechanisms by which monocytes are attracted to bone surfaces may be of fundamental importance to our understanding of bone cell dynamics and bone resorption.

The source of bone used in these experiments was the 19-d fetal rat radius and ulna, which had been previously labelled with ⁴⁵Ca as described previously^{8,9}. These bones resorb in organ culture in response to parathyroid hormone (PTH) and other stimulators of bone resorption^{8,9}. Bones were cultured

Fig. 1 A serial section of a tumour formed from proliferating aflatoxin B₁ treated cell populations in the nude mouse. The tumour was excised after 6 weeks in the mouse, fixed in glutaraldehyde and stained with haematoxylin and eosin. This tissue section reveals that tumour vascularisation has taken place. $\times 120$.

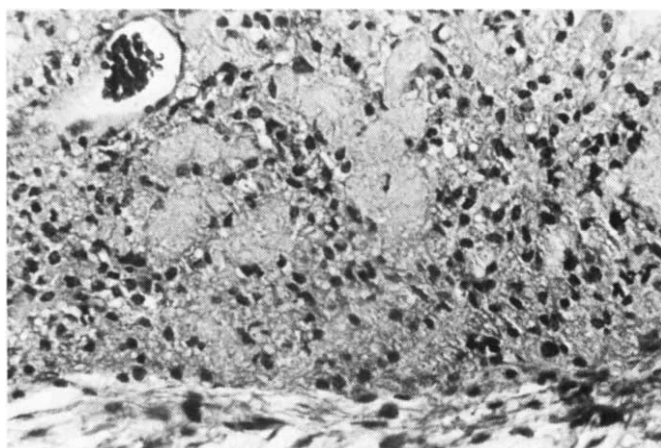


Table 1 Chemotactic responses of human monocytes and neutrophils to resorbing bone, using the Boyden chamber technique

Chemotactic sample	Bone resorption* (% ^{45}Ca release)			Monocyte chemotactic activity†		Neutrophil chemotactic activity†
	Expt A	Expt B	Expt C	Expt A	Expt B	Expt C
Media from PTH (400 ng ml $^{-1}$)-treated bones	65 ± 10‡	94 ± 3‡	83 ± 2‡	165 ± 15‡	61 ± 9‡	16 ± 1
Media from paired bones cultured in control media	27 ± 0.3	59 ± 7	33 ± 4	83 ± 6	27 ± 3	19 ± 2
Control media				86 ± 10	41 ± 5	29 ± 2
Control media + PTH (400 ng ml $^{-1}$)				94 ± 13		
Bacterial factor				140 ± 13‡	69 ± 14‡	102 ± 4‡
Leukotactic factor from zymosan-activated serum					118 ± 9‡	116 ± 6‡

Experiments A and B were carried out using polycarbonate filters (pore size 5 μm) and experiment C with cellulose acetate filters (pore size 0.65 μm). Bones were cultured in BGJ medium supplemented with bovine serum albumin (2 mg ml $^{-1}$). In experiments A and B the mononuclear cell concentration added to the upper compartment of the Boyden chambers was 5×10^6 cells ml $^{-1}$. In experiment C, the starting neutrophil concentration was 2.5×10^6 cells ml $^{-1}$. Bovine PTH (400 ng ml $^{-1}$) was provided by G. Auerbach; $1\alpha, 25(\text{OH})_2 \text{D}$ (10^{-8} M) was provided by Hoffman-LaRoche; PGE $_2$ (10^{-7} M) was from Upjohn.

* Values are means $\pm$ s.e.m. for 4 pairs of bones.

† Chemotactic activity is expressed as the average number of monocytes or neutrophils into the filter per high power field ($\pm$ s.e.m.).

‡ Significantly greater than control media ($P < 0.05$).

for 120 h in BGJ medium (Gibco) supplemented with bovine serum albumin (2 mg ml $^{-1}$) or 5% heat-inactivated fetal calf serum. PTH and other stimulators of bone resorption were added to culture dishes containing two long bones in 0.5 ml medium. Corresponding paired bones from the opposite limb were cultured in control media. Bone resorption was quantified by measuring the percentage of total ^{45}Ca released from the two bones into the medium during the culture period. Media taken from the stimulated and control bones were tested as potential attractants for human monocytes using both the Boyden chamber apparatus¹⁰ and the technique of monocyte chemotaxis under agarose¹¹.

Mononuclear cells were obtained from normal plateletpheresis donations from the Connecticut Red Cross, Farmington¹. The leukocyte-rich buffy coat was separated from red cells and platelets by adding 5 ml 6% dextran and allowing the red cells to sediment by gravity. The buffy coat contained about 85% lymphocytes and 15% monocytes. Few polymorphonuclear leukocytes were present. The cells were suspended in BGJ medium at a concentration of 2.5×10^6 cells ml $^{-1}$ and added to the upper compartments of modified Boyden chambers¹⁰. The Boyden chambers were incubated in an atmosphere of 5% CO $_2$ and air at 37 °C for 4 h, after which the filters were removed, fixed, stained with haematoxylin, and examined by light microscopy (250 $\times$ magnification). Two types of Micropore filters were used, polycarbonate filters with uniform diameter linear pores of 5 μm diameter (Nucleopore) and nitrocellulose filters which have convoluted pores of 8 μm diameter (Schleicher and Schuell). Chemotactic activity was expressed as the number of cells which migrated into the filter in six random microscopic fields.

Table 1 shows the relative migration of mononuclear cells from the upper compartment of the Boyden chambers towards media from control or PTH-treated bones in the lower compartment. PTH in maximal doses caused more than 60% bone resorption as indicated by ^{45}Ca release. Media taken from the PTH-resorbed bones clearly attracted mononuclear cells into the membrane filters compared with media taken from the control bones and control media (Table 1). This effect was not due to PTH because when PTH-containing media which had not been cultured with bone was added to the lower compartment, there was no chemotactic effect (Table 1). In another experiment, PTH was added to media taken from bones cultured in control media, but again there was no increased chemotactic effect compared with control media alone, or control media cultured with bones for 120 h (data not shown).

The chemotactic activity of the resorbed bones was similar to that of two known powerful chemotactic agents, the leukotactic fractions obtained from zymosan-activated serum and the chemotactic factor produced by *E. coli*. Bacterial factor was obtained from overnight culture fluid of rapidly replicating *E. coli* cultures (WHO strain 16). The bacteria were removed by centrifugation and the factor was diluted 1/5 before use. Leukotactic fragments were obtained from serum by incubation with 10 mg zymosan per ml of serum at 37 °C for 1 h. When media from PTH-resorbed bones was added to the upper compartment or to both upper and lower compartments, there was no increase in movement of cells into the filters. Thus, increased movement of human monocytes into the membrane filters in response to resorbed bone media could not be ascribed to increased random cell migration.

The cells which were placed in the upper compartment of the Boyden apparatus at the beginning of the chemotaxis experiments were predominantly lymphocytes (85%). There were a smaller number of monocytes (15%) and polymorphonuclear leukocytes (<1%) (Fig. 1a). On the underside of the filter, only monocytes were observed (Fig. 1b). Thus, these experiments show that the chemotactic effect was directed towards monocytes and not lymphocytes or polymorphonuclear leukocytes. The absence of any effect on polymorphonuclear leukocytes

Table 2 Effect of different mediators of bone resorption on monocyte chemotaxis in Boyden chambers, using nitrocellulose filters with a pore size of 8 μm

Chemotactic sample	Bone resorption* (% ^{45}Ca release)	Chemotactic activity†
PTH (400 ng ml $^{-1}$) bone media	45 ± 8‡	48 ± 2‡
PGE $_2$ (10^{-7} M) bone media	32 ± 2‡	38 ± 2‡
$1\alpha, 25(\text{OH})_2 \text{D}$ (10^{-8} M) bone media	62 ± 4‡	55 ± 3‡
Control bone media	22 ± 1	32 ± 2
Dead bone media	14 ± 0.4	29 ± 2
Bacterial factor		52 ± 3‡

* Values are means $\pm$ s.e.m. for 6 pairs of bones.

† Chemotactic activity is expressed as the mean number of monocytes ($\pm$ s.e.m.) migrating into the filter per high power field (250 $\times$).

‡ Significantly greater than control bone sample ($P < 0.05$).

was confirmed by adding polymorphonuclear leucocytes alone to the upper compartment of the apparatus with resorbed bone media in the lower compartment. Although this PTH-treated bone media was actively chemotactic for monocytes, there was no increased chemotactic effect on polymorphonuclear leucocytes.

In another experiment, bones were incubated with two other humoral stimulators of bone resorption, 1,25-dihydrocholecalciferol and prostaglandin E₂ (Table 2). Media taken from these resorbed bones were also chemotactic for monocytes. There was a correlation between the extent of bone resorption and the chemotactic activity (Table 2). Thus, monocytes are attracted by media taken from resorbed bones independent of the humoral mediator of bone resorption.

The chemotaxis of monocytes towards resorbed bone products was also demonstrated using the technique of chemotaxis under agarose¹¹. In this method the cells migrate under semi-solid media in a tissue culture dish towards the adjacent chemotactic factor. A 10- μ l aliquot of cell suspension (containing 10⁶ cells) was placed in the centre well and four 19-d foetal rat radii and ulnae were placed in each outer well. In some dishes, PTH was added to the agarose-containing medium to stimulate the bones to resorb. Control dishes contained killed bones or unstimulated live bones. At the end of an 18-h incubation period in 5% CO₂ and air at 37 °C, the cells were fixed with absolute methanol followed by formalin, and stained with Wright's stain. Migration was quantified by

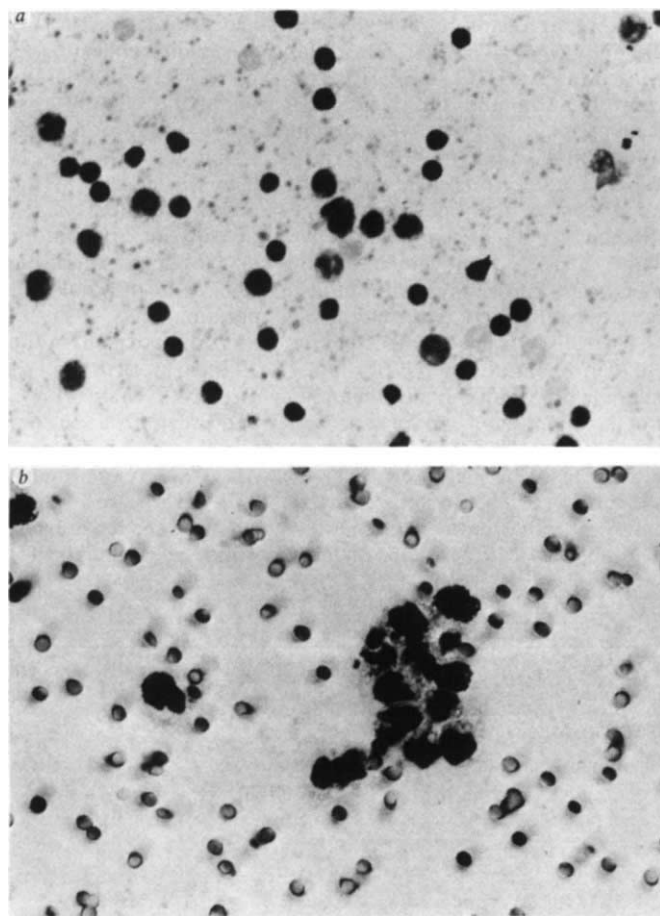


Fig. 1 *a*, Wright's stain of human leukocytes applied to upper compartment of Boyden chamber apparatus (250 $\times$). These cells were predominantly lymphocytes with a small number of monocytes and polymorphonuclear leukocytes. *b*, Wright's stain of cells which migrated through the membrane filter in response to resorbed bone products (250 $\times$). All of the cells observed were monocytes in spite of the mixed cell population added to the opposite (upper) compartment. The small circles are the pores in the polycarbonate filter.

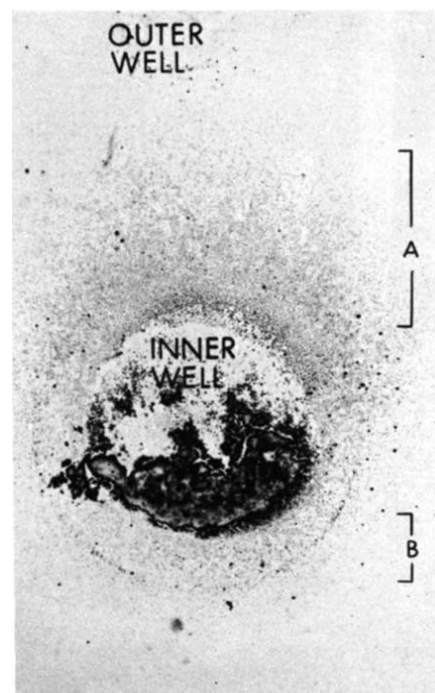


Fig. 2 Pattern of differential migration of human mononuclear cells in response to resorbing bones using the method of chemotaxis under agarose¹¹. Resorbing bones were in the outer well and the leukocytes (10⁶ cells in 10 μ l) were placed in the inner well. PTH (400 ng/ml) was added to the agarose medium. Distance A is the distance the cells migrated from the edge of the inner well towards the resorbing bones. Distance B represents spontaneous migration. The cells which migrated toward the bones in the outer well were monocytes.

projecting the pattern on to a white background and measuring the linear distance the cells had migrated toward the bones. In those dishes in which PTH was present, the bones were visibly resorbed and there was linear movement of the cells towards the wells containing the bones (Fig. 2). There was no elliptical ring of cells in dishes without PTH or in dishes which contained dead bones. The front advancing towards the resorbed bones comprised over 95% monocytes by light microscopic appearance.

The chemical nature of the factor or factors released by bone which is chemotactic for monocytes awaits further study. Small molecular weight factors such as calcium cannot account for these chemotactic effects since medium taken from PTH-treated bones did not lose chemotactic activity after dialysis. It is more likely that a fragment or product of bone matrix digestion is responsible. Postlewaite and Kang¹² have found that collagen-peptide fragments of chick and human skin collagen obtained by digestion with cyanogen bromide and pepsin or collagenase were chemotactic for monocytes but not for polymorphonuclear leukocytes. However, skin collagen differs chemically from bone collagen and whether similar chemotactic peptides are released during the bone resorption process is unknown.

The attraction of monocytes to bone resorbing margins may be of both physiological and pathological importance. Mononuclear cells are often found adjacent to bone-resorbing surfaces. Their role *in vivo* is unknown but it is possible that they participate in the bone-resorbing process because they are capable of resorbing bone *in vitro*^{1,2}. In addition, there is strong circumstantial evidence that osteoclasts, the major bone-resorbing cells, are derived from circulating mononuclear cells³⁻⁷. Thus, whether monocytes are precursors of osteoclasts or are bone-resorbing cells themselves, or both, the control of their movement to active bone margins may be a process of fundamental importance in bone resorption.

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GREGORY R. MUNDY
JAMES VARANI
WILLIAM ORR
MONICA D. GONDEK
PETER A. WARD

Departments of Medicine and Pathology,
University of Connecticut School of Medicine,
Farmington, Connecticut 06032

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Loss of immunoreactivity in long-term bone marrow culture

WE have developed a long-term bone marrow culture system in which proliferation and differentiation of apparently functionally normal haemopoietic stem cells (CFU-S) can be sustained *in vitro* for several months^{1–8}. We now find that genetic resistance^{9,10} or host-versus-graft (H-v-G) reaction, the (possibly) related natural killer (NK) cell activity¹¹ and the ability of cultured cells to reconstitute irradiated allogeneic (non-compatible) recipients (with the subsequent development of G-v-H disease¹²) are all absent from such cultured cells.

The maintenance of stem cells *in vitro* seems to depend on prior establishment of populations of marrow derived adherent cells (including endothelial cells, large, fat-containing cells and macrophages) which we speculate provide a suitable microenvironment necessary for sustained haemopoiesis^{2,3}. When such adherent layers are seeded with freshly isolated syngeneic or allogeneic marrow⁴, the stem cells migrate into the adherent layer and undergo extensive self-renewal, liberating cells into the growth medium⁵. Such cells can be collected and assayed at weekly intervals, and allow monitoring of events occurring in the cultures. Concomitantly with self-renewal, stem cells undergo differentiation to a variety of progenitor cells, including CFU-C (granulocyte/macrophage progenitors) and CFU-M (megakaryocyte precursors)^{1,6,7}. While subsequent development of BFU-E into mature erythroid cells does not occur in the culture system, it maintains maturation of CFU-C and CFU-M into mature granulocytes/macrophages and megakaryocytes respectively^{1,7}. No B- or T-lymphocytes are detected after the first week, although recent work indicates that a precursor cell, 'specific' for lymphopoiesis, is present even after several weeks⁸. This system has now been used to study the mechanisms underlying the phenomenon of bone marrow transplantation resistance.

When limited numbers of bone marrow cells from certain parental mice, for example C57BL/6, are injected into irradiated BDF₁ hybrids (C57BL/6 × DBA/2) a rejection mechanism ensures the killing of these cells within the first 1–2 days^{9,10}. This 'genetic resistance' is determined not by the H-2 antigens, but by non-codominantly inherited haemopoietic histocompatible

Table 1 Maintenance of stem cells (CFU-S) in allogeneic bone marrow chimaeras *in vitro*

Adherent layer	Cells inoculated BDF ₁ :C57BL/6	CFU-S maintenance	
		BDF ₁	C57BL/6
BDF ₁	1:1	> 5 weeks	> 5 weeks
	1:9	> 5 weeks	> 5 weeks
	9:1	> 5 weeks	> 5 weeks
	19:1	> 5 weeks	> 5 weeks
	1:19	> 5 weeks	> 5 weeks

Adherent layers established from BDF₁ femoral marrow¹ were re-inoculated after 3 weeks with 10 ml of growth medium containing different ratios of BDF₁:C57BL/6 bone marrow cells, to a final concentration of 10⁶ nucleated cells per ml. The cultures were then fed at weekly intervals¹ and the non-adherent cells assayed for CFU-S²¹ in BDF₁ and C57BL/6 recipients respectively. It had previously been established that at the cell numbers injected cultured C57BL/6 CFU-S would not form spleen colonies in BDF₁ mice and vice versa.

antigens (present on the surface of primitive haemopoietic cells) and *Ir* genes, which determine the responsiveness. The cell-type involved in the rejection process seems to be bone-marrow derived, and shares properties in common with macrophages¹⁰. Previous work showed that the BDF₁-derived bone marrow adherent layers would support the growth of semi-allogeneic C57BL/6 parental stem cells⁴ and it was suggested that either the effector cell involved in genetic resistance was not present within the adherent layer population, or that the effector cell system had been 'swamped' by inoculation of excessive numbers of target cells. In more recent work, we have shown that a BDF₁ adherent layer will support the growth of very low numbers of semi-allogeneic stem cells. Using published procedures^{1,4} BDF₁ marrow cells were allowed to form an adherent layer for three weeks at which time all the growth medium was removed, and the adherent layer gently washed with growth medium. The cultures were then reconstituted with 10 ml of growth medium containing various ratios of BDF₁:C57BL/6 bone marrow cells, at a final concentration of 10⁶ cells per ml. The results (Table 1) show that irrespective of the cell combination used, there is always extensive proliferation and differentiation of both stem cell populations (in some experiments it has been possible to show proliferation of three or even four bone marrow stem cell chimaeras *in vitro*) and no evidence of rejection of the relatively low numbers of semi-allogeneic stem cells was observed.

When equal numbers of freshly isolated BDF₁ and C57BL/6 bone marrow cells were mixed, and allowed to establish an adherent layer before inoculation with DBA/2 bone marrow cells, stem cell proliferation was also observed—direct mixing of semi-allogeneic or allogeneic marrow cells did not inhibit the capacity of the adherent layer formed to support haemopoiesis, indicating also that the important milieu producing cells are not destroyed in such circumstances.

The clear association between effector cells involved in genetic resistance and natural killer (NK) cells¹¹ led us next to examine NK activity in these cultures (Table 2). The activity in freshly isolated marrow cells was appreciably lower than that seen with spleen cells, but culture of either spleen or bone marrow cells resulted in a rapid decline in activity—falling to levels not significantly above the background within 24 h following spleen cell culture. The loss of NK cell activity in the cultures, and the lack of response against allogeneic marrow grafts, supports the contention that similar effector cells are involved in both phenomena. Interestingly, resistance to certain leukaemia viruses and tumour cells has also been shown to be mediated by cells having characteristics in common with NK cells and host-versus-graft effector cells, suggesting that there may be a common effector cell^{11,14,15}. Yet, in studies with leukaemia virus infection of long-term bone marrow cultures, we have demonstrated the continued maintenance of resistance

Table 2 NK cell activity in normal or cultured spleen and bone marrow cells (BDF₁ mice)

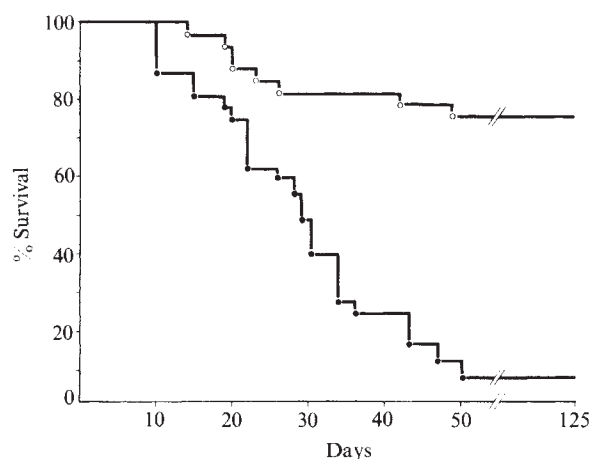
Effector: target cell ratio	Normal spleen Expt		Cultured spleen non-adherent cells 24 h Expt		Cultured spleen non-adherent + adherent cells 24 h Expt		Cultured spleen non-adherent + adherent cells 7 d	Normal bone marrow	Cultured bone marrow 7 d
	1	2	1	2	1	2			
100:1	27.0	30.7	2.8	1.9	1.1	2.3	6.8	12.0	3.3
50:1	13.7	16.7	1.9	1.0	1.2	1.1	3.2	6.5	0.8
25:1	9.4	7.7	1.7	1.1	1.1	1.5	6.0	3.4	0.0

Spleen cultures were established by inoculating 10^8 spleen cells into culture flasks containing 10 ml of growth medium¹. Various times later the non-adherent cells were collected and assayed separately, or as a mixture, with the adherent cells for NK activity. Bone marrow cultures were established in the conventional way¹ and assayed 7 d after inoculation of the second bone marrow population. NK cell activity was measured by the cytotoxicity of spleen or bone marrow cells against ⁵¹Cr labelled K562 human CML in blast crisis target cells. 10^4 K562 and varying numbers of effector (marrow or spleen) cells were incubated overnight and the ⁵¹Cr release from the cells measured according to published procedures²². Similar results were obtained when YAC-1 target cells were used.

in vitro^{16,17}. The cell type involved in this type of resistance, therefore, seems to be distinct from NK cell or H-v-G activity.

The possibility that cultured stem cells could be used to reconstitute the haemopoietic system of irradiated allogeneic mice, without the development of H-v-G disease, was indicated by our finding that T lymphopoiesis was not maintained in the cultures (since T lymphocytes are the effector cells involved in the H-v-G response¹⁸). Figure 1 demonstrates that 90% of BDF₁ mice, injected with normal C57BL/6 bone marrow cells, died within 70 d post reconstitution. On necropsy the mice had grossly enlarged spleens indicative of G-v-H disease^{12,19}. At this time only 20% of mice reconstituted with cultured C57BL/6 stem cells had died—with most of the deaths occurring in the first 4 weeks after reconstitution. At 120 d after reconstitution, some mice from this group were sacrificed, and the femoral marrow cells were assayed for CFU-S using irradiated BDF₁ and C57BL/6 mice as recipients. Spleen colonies developed only in the latter strain, showing that the cultured C57BL/6 marrow cells had indeed reconstituted the original BDF₁ recipients. Injection of cultured bone marrow cells, therefore, gives a situation analogous to that seen following reconstitution of irradiated mice with marrow cells which have been selectively depleted of T lymphocytes by immunological techniques^{18,20}.

Fig. 1 Survival of irradiated BDF₁ mice reconstituted with normal (●) or cultured (○) C57BL/6 bone marrow cells. C57BL/6 bone marrow adherent layers, established for three weeks, were 're-charged' with 10^7 C57BL/6 bone marrow cells. The cultures were fed weekly by demi-depopulation. Two and three weeks later, the cells were collected and 5×10^6 – 10^7 cells containing approximately 750–1,500 haemopoietic stem cells or CFU-S²¹ were injected into irradiated (800 rad X rays) BDF₁ mice. Control mice received 5×10^6 – 10^7 freshly isolated (not cultured) C57BL/6 bone marrow—containing approximately equal numbers of CFU-S. Each group comprised 40 animals.



The significance of the present observations lies in the possibilities of establishing human cultures using allogeneic bone marrows and the potential of exploiting such cultures for the production of stem cells for marrow transplantation purposes (without the disadvantages of developing G-v-H disease). Furthermore, the absence of mature B, T and NK cells, in the murine system is allowing work on the genesis of these cells from the pluripotent haemopoietic stem cell.

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T. M. DEXTER
E. SPOONER

Paterson Laboratories,
Christie Hospital and Holt Radium Institute,
Manchester, UK

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Hepatic glucocorticoid receptors and the H-2 locus

VARIOUS inbred strains of mice exhibit different degrees of susceptibility to both spontaneous and glucocorticoid-induced cleft palate^{1,2}, and specific genetic and biochemical factors that may be responsible for these differences have been identified. At the genetic level, variations at the H-2 locus have been associated with susceptibility to glucocorticoid-induced cleft palate^{3,4}, while at the biochemical level, evidence suggests that numbers and characteristics of glucocorticoid receptors in murine embryonic facial mesenchyme cells differ in the various

strains. Specifically, Salomon and Pratt showed that facial mesenchyme cells cultured from fetuses of the A/J strain (which is highly susceptible to glucocorticoid-induced cleft palate) contain twice as many glucocorticoid receptors as do such cells from C57BL/6J, a low susceptibility strain⁵. They also reported that the glucocorticoid receptor of the A/J strain has a lower affinity for dexamethasone ($K_D = 1.7 \times 10^{-8}$ M) than does that of the C57BL/6J strain ($K_D = 7.9 \times 10^{-9}$ M) (ref. 5). In an attempt to relate steroid receptor activity to *H-2* haplotype, Goldman *et al.* used ³H-cortisol and isoelectric focusing to study glucocorticoid receptors in facial mesenchyme cells of these and several other strains⁶. They concluded that "a product of a gene in or near the *H-2* locus seems to be the glucocorticoid receptor, the level of which must be increased for a congenital malformation to occur". Such conclusions must be questioned, however, because the experimental procedure required microelectrofocusing of isolated protein-cortisol complex for 40 h. Receptor binding is reversible, and in the absence of free steroid, the half life of the receptor-cortisol complex is only about 1 h at 0 °C (ref. 7). It is unlikely that a peak of radioactivity detected by isoelectric focusing after 40 h represents binding to a specific receptor. Thus an association between *H-2* haplotype and glucocorticoid receptor activity has not been established definitively. However, the possibility that *H-2* might determine hormone receptor levels or characteristics is exciting. We have explored the relationship between *H-2* and glucocorticoid receptors by examining the level of specific glucocorticoid-binding activity in liver cytosols prepared from different mouse strains.

Liver is suitable for such studies because liver cytosol preparations tend to have a relatively stable glucocorticoid-binding activity^{8,9}, while unbound glucocorticoid receptor molecules undergo rapid, temperature-dependent inactivation in most other cell-free systems. The liver is also well characterised as a target of glucocorticoid action, and *H-2* is expressed in this organ¹⁰. Thus an effect of *H-2* haplotype on the amount or affinity of specific glucocorticoid binding should be observed in this tissue. We examined specific glucocorticoid binding in the two strains studied by Salomon and Pratt, A/J (high susceptibility) and C57BL/6J (low susceptibility), as well as in the two *H-2* congenic lines A.BY and B10.A.

Because glucocorticoid-receptor inactivation is greatly accelerated at elevated temperatures⁷, we took care to prepare and maintain the liver cytosol at 0 °C. Adult males were killed by decapitation. Livers were removed and chopped on glassine paper over dry ice, weighed and transferred to a chilled homogeniser. Four volumes of ice-cold buffer (10 mM Tris, pH 7.35, 0.1 mM EDTA) were added and the tissue was homogenised on ice with nine strokes of a motor-driven Teflon pestle. The homogenate was centrifuged at 35,000g for 30 min, the supernatant was removed and assayed for glucocorticoid-binding activity.

Figure 1 shows binding of ³H-TA to the macromolecular component of liver cytosol as a function of free steroid concentration for the A/J and C57 strains. Nonspecific binding (binding of ³H-triamcinolone acetonide (TA) in the presence of an excess of cold dexamethasone) is the same in both strains, whereas total binding is significantly higher in A/J. Specific binding (total less nonspecific, upper inset, Fig. 1) is about twice as high in A/J as it is in C57. Scatchard plots of the data (Fig. 1, lower inset) suggest a single class of high-affinity binding sites. The initial slopes, representing specific binding, are approximately equal, giving an apparent K_D of 1.8×10^{-8} M for both the A/J and C57 strains. Experimentally determined values for specific binding and apparent dissociation constants can be affected significantly by inactivation of the unbound receptor. Rates of receptor inactivation must be similar in cytosol preparations from the two strains before values for dissociation constants and specific binding can be usefully compared¹¹. Figure 2 shows the decline of specific binding activity observed when liver cytosol is incubated in the absence of free steroid for increasing periods. Rates of inactivation of unbound receptors

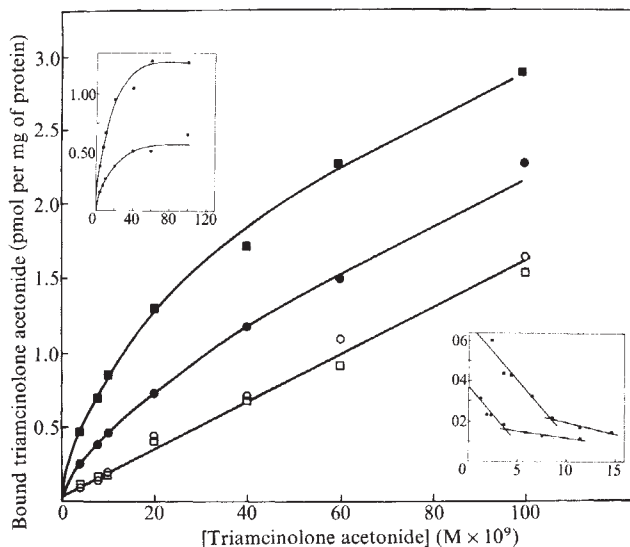


Fig. 1 Binding of ³H-TA presented as a function of free steroid concentration. Samples of 0.2 ml of liver cytosol were incubated in a total volume of 0.5 ml for 22 h at 0 °C with various concentrations of ³H-TA (21.6 Ci mmol⁻¹) in the presence of vehicle or a high concentration (5×10^{-5} M) of competing, nonradioactive dexamethasone. The bound steroid was separated from the free compound by passage through columns (1 cm × 22 cm) of Sephadex G-25 with an elution buffer of 0.01 M Tris (pH 7.35), 0.04 M KCl. The columns were run at 4 °C. Fractions (1 ml) were collected and the macromolecular peak was identified by the presence of blue colour resulting from the addition of two drops of a solution of blue dextran to each sample immediately prior to application to the column. Each point represents the binding expressed as pmol bound per mg of protein. Binding in the presence of vehicle: ■, A/J; ●, C57BL/6J; binding in the presence of nonradioactive dexamethasone (nonspecific binding): □, A/J; ○, C57BL/6J. The upper inset shows specific binding (total minus nonspecific) for each strain. Bound TA (pmol per mg of protein) is plotted against unbound TA ($M \times 10^9$). The lower inset presents Scatchard plots of the binding in the presence of vehicle. The ratio of bound to unbound TA is plotted against bound TA ($M \times 10^{10}$).

are the same in both strains. These rates and the observed levels of glucocorticoid binding were not affected by dithiothreitol.

To determine whether or not differences in glucocorticoid-receptor activity observed in the two strains are mediated by the *H-2* locus, activities of glucocorticoid receptors in the two congenic lines, A.BY and B10.A, were determined. A.BY has the *H-2^b*-region of the C57 strain placed on the A genetic background, while B10.A has the C57 background, but the A/J *H-2^a*-region. The results are shown in Table 1. The mean specific binding capacities in the four strains are significantly different ($P < 0.002$), however the difference is due to genetic background ($P < 0.001$) and not *H-2* ($P = 0.99$). There is some possibility of an *H-2* × background interaction effect ($P = 0.10$), which in this case would mean that the effect of genetic background on levels of glucocorticoid receptors is more pronounced in the presence of the *H-2^b*-haplotype than in the presence of the *H-2^a*-haplotype.

Thus we have shown that (1) rates of glucocorticoid-receptor inactivation are the same in both A/J and C57BL/6J mice; (2) affinities of the receptors for TA are the same in both strains; (3) A/J has approximately twice as many glucocorticoid receptors as C57BL/6J, and (4) this difference is not determined by variations at the *H-2* locus.

It is interesting to compare our results with what is known about spontaneous and glucocorticoid-induced incidence of cleft palate in these strains. The incidence of cortisone-induced cleft palate in A/J is 100%, in B10.A, 81% and C57, 21% (ref. 4), which corresponds in rank order to the observed levels of

Table 1 Relationship of specific glucocorticoid-binding capacity to *H*-2 and genetic background

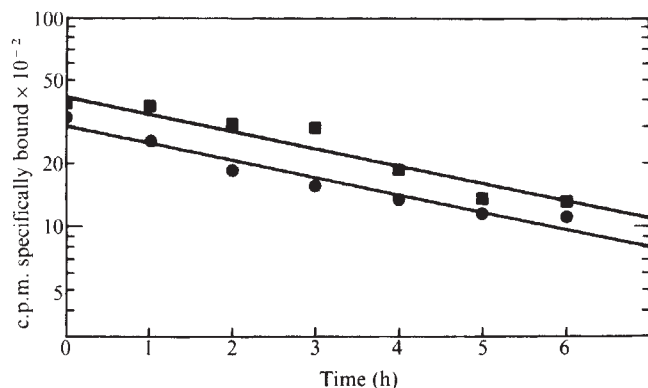
Background	Haplotype	
	<i>H</i> -2 ^a	<i>H</i> -2 ^b
A	A/J	A.BY
	1.29 ±0.11 (10)	1.49 ±0.16 (9)
C57Bl	B10.A	C57Bl
	0.99 ±0.10 (9)	0.83 ±0.09 (10)

Incubations (0.5 ml) containing liver cytosol and 6×10^{-8} M 3 H-TA in the presence of vehicle or nonradioactive competing dexamethasone were maintained for 3 h at 0°C. Specifically bound steroid was determined as outlined in the legend to Fig. 1. The numbers represent mean specific binding (pmol of 3 H-TA bound per mg of cytosol protein) ± standard error for each of the four strains. Specific binding was determined in duplicate for each animal. The number of animals used to determine each mean is given in parentheses.

glucocorticoid receptors in these strains. The steroid-induced frequency of cleft palate in A.BY was not studied, but in our laboratory it has the highest spontaneous incidence of cleft palate of all four strains (19, 3, 0 and 0% for A.BY, A/J, B10.A and C57, respectively). Because A.BY also has the highest level of steroid receptors, glucocorticoid receptor levels may be an important determinant of susceptibility to spontaneous cleft palate in mice.

Although susceptibility to glucocorticoid-induced cleft palate has been shown to be partially controlled by *H*-2 haplotype, these data demonstrate that the level of hepatic glucocorticoid receptors is determined by other genetic differences between the strains. Whether the factors controlling the levels of binding activity in liver reflect those that operate in the mesenchymal cells of the fetal palate is of course open to question. We observe the same difference in glucocorticoid binding capacity in liver as was seen by Salomon and Pratt in cultured fetal facial mesenchyme cells. This strongly suggests that the observed genetic control of receptor levels is not tissue specific. Our data, unlike theirs, do not suggest different affinity constants for the receptors in the two strains. Differences in binding capacity between the strains could arise from differences in the rates of receptor synthesis or degradation, or as has been suggested¹²⁻¹⁴, differences in rates of receptor activation and/or inactivation. Given the equivalent rates of inactivation presented in Fig. 2, the latter does not seem to be likely in this case.

Fig. 2 Unbound-receptor inactivation at 0°C. Liver cytosol was incubated at 0°C in the absence of labelled steroid for various times, after which saturating levels (6×10^{-8} M) of 3 H-TA were added in both the presence and absence of an excess of cold dexamethasone. Specifically bound steroid was determined as described in the legend to Fig. 1. ■, A/J; ○, C57.



Although *H*-2 is not the locus of prime importance in determining levels of hepatic glucocorticoid receptors in these strains, it may still be possible, using recombinant inbred lines, to identify specific loci which do regulate levels of glucocorticoid receptors. It may be particularly informative if lines with high levels of receptors and low susceptibility to glucocorticoid-induced cleft palate could be found.

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MARTIN S. BUTLEY
ROBERT P. ERICKSON
WILLIAM B. PRATT

Department of Human Genetics,
Department of Pharmacology,
University of Michigan Medical School,
Ann Arbor, Michigan 48109

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Effect of L-triiodothyronine on (-) 3 H-dihydroalprenolol binding and cyclic AMP response to (-)adrenaline in cultured heart cells

MANY physiological responses of the cardiovascular system to thyroid hormone seem to reflect altered sympathetic activity. The effects of thyroid hormones on the heart are very similar to those induced by catecholamines and the possible interrelationship of these substances on myocardial function has been explained by an interaction at various levels. These include effects on altering catecholamine release from the sympathetic nervous system^{1,2}, and modifying plasma catecholamine concentrations^{3,4}, as well as tissue catecholamine content⁵ and responses⁶⁻⁸. Although several investigators have attempted to define an action of thyroid hormone and its possible role in potentiating the catecholamine response, the mechanism responsible for this modification has not been clarified. We have reported previously that physiological concentrations of L-triiodothyronine (T_3) stimulate the rate of glucose metabolism in cultured heart cells prepared from newborn rats⁹. We now demonstrate here that cultured heart cells retain the characteristic of response to β -adrenergic stimulation and that this response seems to be influenced by T_3 .

The heart cells were prepared as described previously^{9,10}. The trypsinised cells were inoculated into 25-cm² plastic T-flasks (Falcon) at densities of approximately 200,000 cells cm⁻² and then grown in Ham's medium (Gibco) supplemented with 10% fetal calf serum (FCS) at 37°C (95% air, 5% CO₂).

Catecholamine action seems to involve activation of adenylate cyclase, which then leads to an increase in the intracellular concentration of cyclic AMP¹¹. Therefore we examined the cyclic AMP response to (-)adrenaline and the inhibitory effect

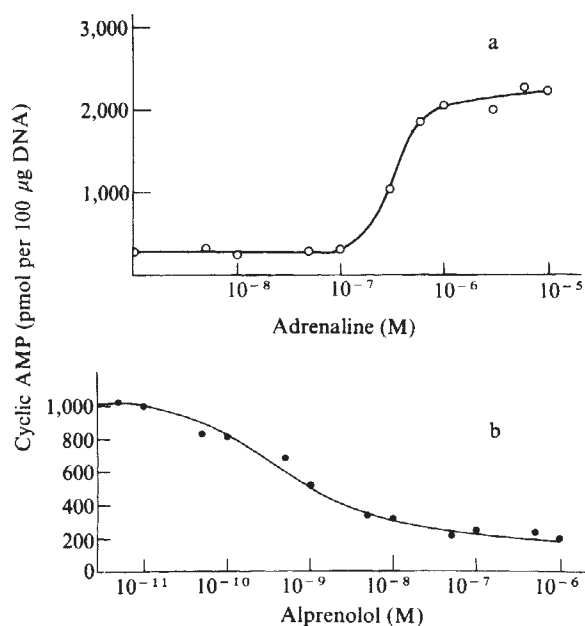


Fig. 1 Dose-response relationship of *a*, (-)-adrenaline stimulation of cyclic AMP production and *b*, (-)-alprenolol inhibition of the (-)-adrenaline-induced cyclic AMP response in the cultured heart cell. *a*, After 5 d growth of the culture, the culture flasks were partially submerged in a water bath at 37 °C for 2 min, to enable there to be precise temperature control during the experiment. The upper surface of the flasks was then opened with a haemostat. Various concentrations of (-)-adrenaline bitartrate (Sigma) dissolved in Ham's F-10 medium was added to the culture media. After a 2-min incubation, the media were aspirated and 1 ml of 10% ice-chilled trichloroacetic acid (TCA) was added. The cellular material was then removed using a rubber policeman at 4 °C and the samples were centrifuged at 2,000g for 15 min. The cell TCA precipitates were analysed for DNA by the method of Burton²⁰, and the supernatant was used for cyclic AMP assay by a modification of competitive binding method of Gilman²¹. The concentration of (-)-adrenaline which induced a half-maximal cyclic AMP response was 0.2 µM; above a concentration of 1 µM, (-)-adrenaline induced a maximal cyclic AMP response. *b*, Blocking effect of (-)-alprenolol on (-)-adrenaline-induced cyclic AMP production in a dose-related fashion. Various concentrations of (-)-alprenolol were added to the culture for 30 min before the addition of (-)-adrenaline (1 µM). The concentration of (-)-alprenolol which gave half-maximal inhibition was approximately 0.27 µM.

of the catecholamine antagonist, (-)-alprenolol, on the heart cell cultures. (-)-Adrenaline bitartrate at 1 µM, induced a sixfold increase in intracellular cyclic AMP within 2 min of incubation, which subsequently declined to a plateau higher than the basal level for up to 30 min. The intracellular cyclic AMP in cultured heart cells responds to (-)-adrenaline in a dose-related fashion; at concentrations greater than 1 µM, (-)-adrenaline induces a maximal response (Fig. 1*a*). This response of cyclic AMP can be antagonised effectively by (-)-alprenolol, which is a potent β -adrenergic antagonist, also in a dose-dependent fashion (Fig. 1*b*). The effect of preincubation of the cells with various concentrations of (-)-alprenolol for 30 min before induction of cyclic AMP by (-)-adrenaline was studied. (-)-Alprenolol at concentrations greater than 1 µM completely inhibited the adrenaline-induced cyclic AMP response, indicating that the cultured heart cells possess a biologically responsive β -adrenergic receptor.

It is generally felt that the physiological action of catecholamine agonists which result in an increase of intracellular cyclic AMP are mediated by a β -adrenergic receptor¹². Binding sites with the specificity of β -adrenergic receptor determined by (-)³H-dihydroalprenolol binding have been identified in avian

and amphibian erythrocytes¹³, human lymphocytes¹⁴, and canine myocardium¹⁵. We have also demonstrated that specific binding of (-)³H-dihydroalprenolol to cultured heart cells was very rapid at 37 °C, attaining equilibrium within 5 min. This binding can also be displaced equally rapidly by catecholamine agonists and antagonists.

We have examined the influence of T₃ on β -adrenergic responses, by measuring (-)³H-dihydroalprenolol binding and the cyclic AMP response to (-)-adrenaline. The heart cells were grown first in regular growth medium and then this was replaced with Ham's F-10 medium supplemented with 10% hypothyroid calf serum. The cells remained in the second medium for 24 h, in order to deplete thyroid hormone. The culture medium was then

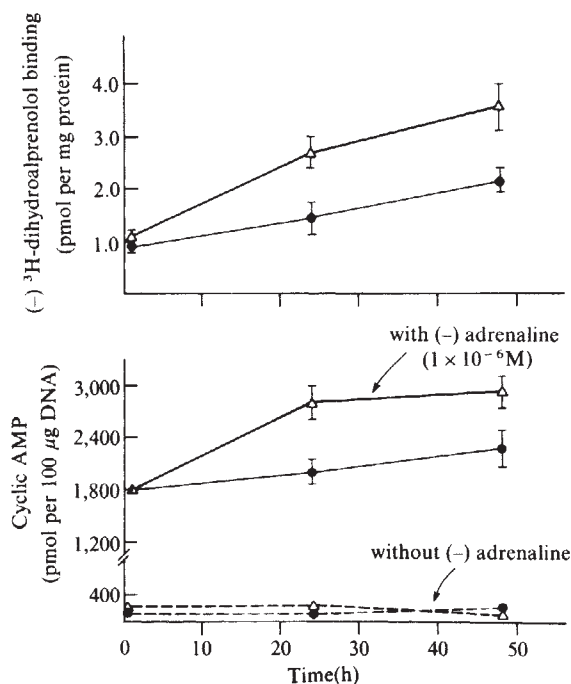


Fig. 2 Effect of L-triiodothyronine on (-)³H-dihydroalprenolol binding (upper), cyclic AMP response (-)-adrenaline (lower). The heart cell cultures were prepared as described in the text. T₃ at 5 nM was added to one group of flasks. At the times indicated, (-)³H-dihydroalprenolol binding and the cyclic AMP response to (-)-adrenaline (1 µM) were measured. For study of (-)³H-dihydroalprenolol binding, the cells were scraped off the flasks with a rubber policeman and then washed twice with 10 ml of 0.145 M NaCl by centrifugation. The cells were suspended in 50 mM Tris-HCl buffer (pH 7.4 at 25 °C) containing 10 mM MgCl₂. Binding studies were carried out by incubating the cells (equivalent 12–18 µg of cell protein per tube) at 37 °C with 80 nM of (-)³H-dihydroalprenolol (NEN, 32.5 Ci mmol⁻¹) in a total volume of 150 µl of incubation buffer. This concentration of (-)³H-dihydroalprenolol gives maximal binding in the culture heart cells. The incubation was terminated by addition of 2 ml of ice-chilled buffer solution, followed by rapid vacuum filtration through Whatman GF/C glass fibre filters with a Millipore sampling manifold. The filters were then washed with 12 ml of ice-chilled buffer and were dried overnight at room temperature. The samples were counted with a Beckman liquid scintillation spectrometer with Aquasol solution (NEN). Nonspecific binding was determined by measuring the amount of radioactivity retained in the presence of a 10,000-fold excess of non-radiolabelled (-)-alprenolol (Sigma). The specific binding illustrated represented the total minus the nonspecific binding. An aliquot of cell suspension from each experiment was analysed for DNA content by the method of Burton²⁰ and cellular protein by the method of Lowry *et al.*²². The cellular protein-DNA ratio was not affected by thyroid hormone during the period of experiments. After incubation for 24 h, T₃ induced a significant increase in the (-)³H-dihydroalprenolol binding and in the cyclic AMP response to (-)-adrenaline. Δ, With T₃ (5 × 10⁻⁹ M); ●, without T₃.

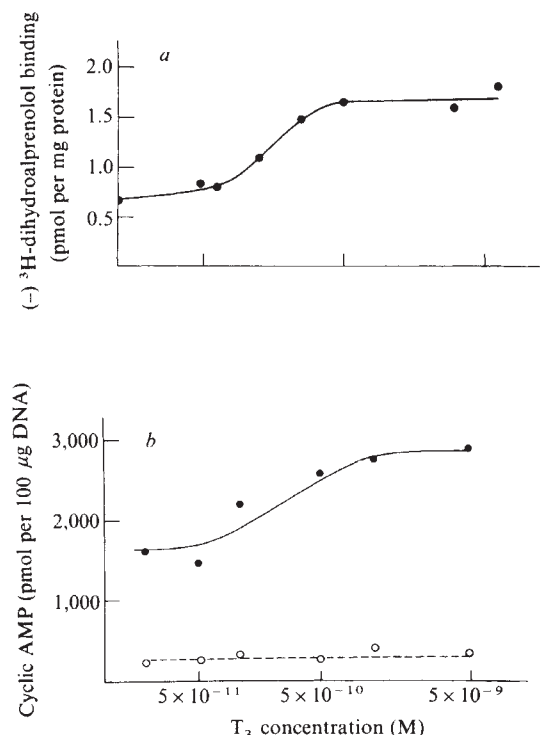


Fig. 3 Effect of T₃ on *a*, (-)-³H-dihydroalprenolol binding and *b*, (-)adrenaline-induced cyclic AMP response. The cultured heart cells were incubated with medium containing various concentrations of T₃ for 24 h and the (-)-³H-dihydroalprenolol binding (*a*) and cyclic AMP response to 1 μM of (-)adrenaline (*b*), were studied simultaneously. T₃ affected both responses in a dose-related fashion. T₃ alone seemed to have no effect on the intracellular cyclic AMP level. ●, With 1 × 10⁻⁶ M (-)adrenaline; ○, without (-)adrenaline.

altered a third time, by adding 5 nM of T₃ to one group of flasks. (This concentration of T₃ has been shown to induce the maximal rate of glucose metabolism in cultured heart cells⁹ and growth hormone production in cultured GH₁ cell system¹⁶, a well established cell-culture system which is responsive to physiological concentration of thyroid hormone.) After 24 h of incubation, T₃ induced a significant increase of (-)-³H-dihydroalprenolol binding on culture heart cells (Fig. 2). The cyclic AMP response to (-)adrenaline was similarly increased by T₃.

We next examined the influence of T₃ concentrations on (-)-³H-dihydroalprenolol binding and the cyclic AMP response to 1 μM (-)adrenaline. As shown in Fig. 3*a*, (-)-³H-dihydroalprenolol binding was increased by thyroid hormone in a dose-dependent fashion. The cyclic AMP response to (-)adrenaline was also enhanced by thyroid hormone in an identical fashion (Fig. 3*b*). The concentration of T₃ (0.3 nM) which resulted in a half-maximal stimulation of cyclic AMP by (-)adrenaline and a half-maximal increase in (-)-³H-dihydroalprenolol binding is virtually identical. Cells incubated with 5 nM of T₃ showed almost a twofold increase in (-)-³H-dihydroalprenolol binding.

L-Triiodothyronine does not affect cell growth as reflected by total cellular DNA or protein content of the cultured cells⁹. The increase in (-)-³H-dihydroalprenolol binding therefore seems to be influenced selectively by T₃ and to be independent of total cell protein. The possibility cannot yet be excluded that T₃ may induce an alteration in membrane structure which cannot be measured in this study¹⁹. Martin and co-workers reported the existence of a β-adrenergic receptor in 13-d-old fetal rat hearts¹⁷. Here we have demonstrated that the β-adrenergic response of cardiac tissue can be maintained in dispersed cultured condition and can be influenced by thyroid hormone in a dose-related fashion. Thyroid hormone has recently been

reported to increase the β-adrenergic receptor in rat myocardium¹⁸. The exact mechanism by which thyroid hormone regulates the β-adrenergic response requires further investigation.

JIR S. TSAI
A. CHEN

Department of Medicine,
New York University Medical Center,
550 First Avenue,
New York, New York 10016

Received 23 May; accepted 12 July 1978.

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Possible plasticity in the rat superior colliculus

THE potential for plasticity of the developing mammalian visual system has been the subject of several investigations^{1,2}. I have now explored the plastic relationship between different sensory modalities. The rodent superior colliculus was chosen as the model as it receives overlapping visual, somatic and auditory inputs^{3,4}. Electrophysiological comparison of dark-reared and normal rats suggests that the rat's superior colliculus is susceptible to effects of sensory deprivation during its postnatal development.

It has been shown in the mouse³ and the hamster⁴ that many neurones in the intermediate and deep layers of the optic tectum receive nonvisual sensory inputs. They respond either to somatic stimuli from fur or whiskers on the contralateral half of the body or to complex sounds from the contralateral side. Some of these cells also receive visual inputs and thus have a bimodal response; more rarely, trimodal cells responding to all three sensory modalities are also encountered. Further, for cells in any one electrode penetration that is nearly normal to the collicular surface, there is general topographical correlation between all the visual, somatic and auditory receptive fields.

In my study using black-hooded rats, I have compared normally reared (12 h light/dark cycle) and dark-reared rats for the relative abundance of the different types of single units in the superior colliculus. I adopted a double-blind procedure, in which the rearing condition of each animal was not revealed to me until I had completed recordings on all the rats. Twenty-nine rats (15 normal and 14 dark-reared), all approximately 20 weeks old and weighing about 300 g, were used in random order. Light deprivation was achieved by rearing the animals in a dark room and the only light they could have received until the time of recording was a few minutes of a red photographic light (<0.1 lm⁻²) during cleaning of cages.

Table 1 Distribution of sensory cell types in the deeper layers of the superior colliculus of normal and light-deprived rats

	Visual	Somatic	Auditory	Visual/ somatic	Bimodal neurons Visual/ auditory	Somatic/ auditory	Trimodal neurons (visual/ somatic/ auditory)	Total
Normal rats	104 (46.2%)	85 (37.8%)	9 (4%)	18 (8%)	3 (1.3%)	3 (1.3%)	3 (1.3%)	225 (100%)
Light-deprived rats	56 (24.5%)	140 (61.1%)	24 (10.5%)	2 (0.9%)	0	7 (3.1%)	0	229 (100%)

The basic operating and recording techniques were similar to those used by Tiao and Blakemore⁴ for the hamster. Glass-coated tungsten microelectrodes with a tip length of 15–20 μm were used and single units were identified by the usual criteria⁵. As the superficial layers of the tectum are densely packed with visual cells even in the dark-reared rats, I have attempted to include in my count cells from intermediate and deep layers only. The occurrence of changes⁴ in the response properties to visual stimuli during an electrode penetration and prior histology indicated the approximate limit to the superficial layers to be 350 μm below the collicular surface. However, all cells encountered below 200 μm were included in the count. The surface of the superior colliculus was identified during the nearly surface-normal penetration by a characteristic change in the background noise, which responded to visual stimuli in the correct part of the visual field with a brisk hiss. The penetration was always continued to a depth of 2,200–2,500 μm , which was at least 500 μm beyond the last cell responding to a sensory stimulus. Histological reconstruction of the electrode tracks in some of the animals confirmed the reliability of our electrophysiological criteria for identifying the superior colliculus. All recordings were restricted to the parts of the superior colliculus whose visual field representation was between 40° and 80° from the midline, with the blind axis monitored at 70°. This was necessary because, towards the caudal end of the colliculus, representing the temporal end of the visual field, there is greater nonvisual input even in the normal rat, and towards the rostral end of the colliculus, representing the nasal end of the visual field, there is greater visual input even in the dark-reared rat. When tested ophthalmologically before recording, none of the rats used showed any obvious difference from the controls.

Table 1 shows the pooled results from 42 penetrations in 15 normal rats and 40 penetrations in 14 dark-reared rats. It can be readily seen that the ratio of nonvisual cells to visual ones has increased markedly in the light-deprived animals. Further, there is an almost total absence of multimodal cells with visual inputs in these animals. The increase in the number of nonvisual cells can be either a relative one due to functional or anatomical dropping out of the visual terminals or may reflect an active take-over of collicular neurones by nonvisual sensory inputs. I have attempted to resolve this question by comparing the mean distribution of cell types per penetration between the two groups of rats. It can be seen from Table 2 that the mean number of cells in a single penetration having visual input has significantly (two-tailed test: $P < 0.001$) decreased in the dark-reared rats. Further, the mean number of cells with nonvisual sensory inputs

has significantly ($0.001 < P < 0.01$) increased, suggesting an active process. This absolute increase in the mean number of neurones responding to nonvisual stimuli also makes it less likely that the reduction in the number of visually driven cells is due to any shrinkage of these cells in the light-deprived rat. However, the contribution of any retinal effects of light deprivation⁶ to the reduction in the number of recordable visual cells is an open issue.

It may not be too speculative to suggest that, at birth, the sensory inputs to the superior colliculus from the different modalities are in a state of competition and in the absence of normal stimulation in one of these channels, the other modalities may come to influence a larger proportion of cells. It does not, however, follow that the discriminative ability of the somatic and auditory systems in the light-deprived rat are, compensatively, better than, or even as good as that in the normal rat. The postnatal visual input may have a directive role in the development of the normal properties of the nonvisual and multimodal neurones of the optic tectum. In its absence, the somatic and auditory systems may in fact be permanently impaired.

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T. R. VIDYASAGAR

Visual Sciences Laboratory,
Dept of Ophthalmic Optics,
University of Manchester,
Institute of Science and Technology,
PO Box 88, Manchester, UK

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Horizontal cells influence membrane potential of bipolar cells in the retina of the turtle

BIPOLAR CELL responses to illumination of the surround have been thought to be mediated by horizontal cells^{1,2}. However, it has been shown recently that the responses of bipolar cells to stimulation with an annulus of light are not suppressed by pharmacological blockade of horizontal cell activity, so alternative mechanisms for the surround effect have been proposed³. The present experiments were performed to establish whether bipolar cells are in fact driven by horizontal cells, and whether the properties of such hypothetical interaction can account for the surround responses of bipolar cells.

Two intracellular microelectrodes were used simultaneously: one to pass current through the membrane of a horizontal cell, and the other to record the membrane potential of an adjacent

Table 2 Mean distribution of cell types per electrode penetration in the superior colliculus of normal and light-deprived rats ($\pm$ s.d.)

	Cells with visual inputs*	Cells with nonvisual sensory inputs†
Normal rats	3.0 $\pm$ 1.3	2.9 $\pm$ 1.9
Light-deprived rats	1.5 $\pm$ 1.0	4.3 $\pm$ 2.1

* Includes multimodal cells with visual inputs.

† Includes multimodal cells with nonvisual sensory inputs.

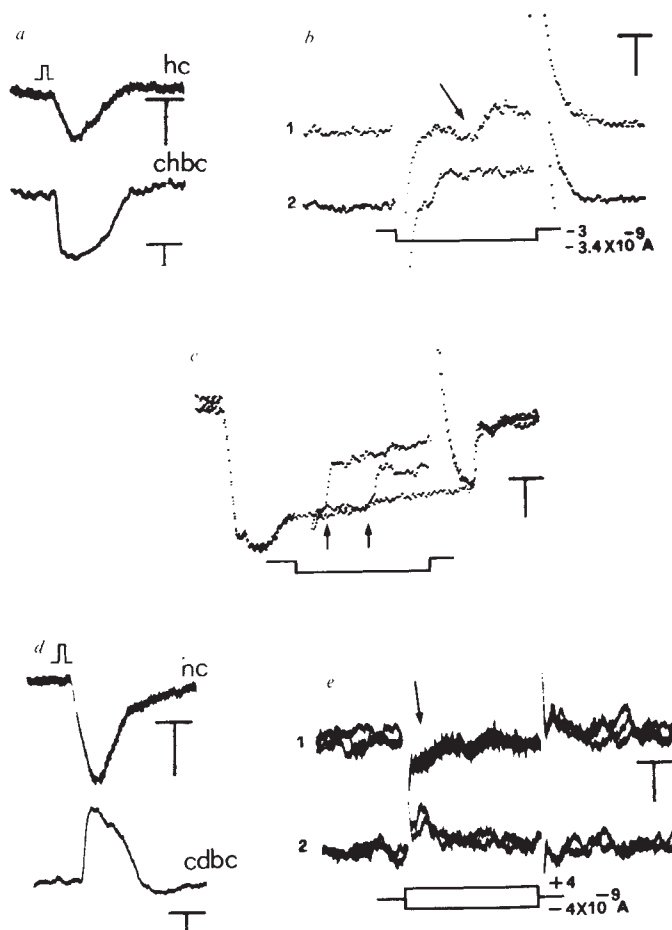


Fig. 1 Simultaneous recordings from L-type horizontal (hc) and bipolar cells. Bipolar cells were centre-hyperpolarising (c.h.b.c) in *a* and centre-depolarising in *d* and *e* (c.d.b.c). *a*, Photoresponses to brief illumination (25 ms, as indicated upper left) in the form of a spot, of 200 μm radius centred on the electrodes penetration site. The light source ($2 \times 10^5 \text{ erg cm}^{-2} \text{ s}^{-1}$, with wavelength between 400 and 800 μm) was attenuated by 3.6 log units. Note that the hc response is 25% of its maximal amplitude with bright light, while the c.h.b.c response has already reached the amplitude saturation level. *b*, Computer-averaged recordings from the same c.h.b.c as in *a*, in darkness. Each record represents the average of 8 sweeps. The lower line represents the duration of the horizontal cell artificial polarisation obtained with the current intensity indicated by the numbers at the right. The arrow in 1 points to the onset of the depolarising response of c.h.b.c. *c*, Computer-averaged recordings from the same c.h.b.c as in *a*. Three superimposed averages of two photoresponses each, recorded without and with artificial horizontal cell hyperpolarisation, whose duration is indicated by the lower line. The arrows point to the onset of the c.h.b.c depolarising responses to current injections of the same intensities as in *b*. A spot of 500 μm radius was used for illumination. Duration of illumination is indicated approximately by the onset and the recovery phase of the c.h.b.s membrane potential. Light intensity as in *a*. *d*, Responses from a horizontal cell (hc) and a bipolar cell (c.d.b.c) to brief illumination (25 ms, as indicated at the upper left) in the form of a spot of 200 μm radius. Light attenuation was -3.6 log units. *e* 1, Superimposed recordings, from the same c.d.b.c as in *d*, of the hyperpolarising responses (arrow) to artificial hyperpolarisation of the horizontal cell; *e* 2, depolarising c.d.b.c responses to artificial depolarisation of horizontal cell. Duration of current injection is indicated by the lower line. Numbers to the right refer to the intensity of injected current. Time and voltage calibrations (100 ms, 5 mV) are indicated by the horizontal and vertical lines, respectively, shown as T symbols, at the right of each recording, from *a* to *e*.

bipolar cell. The methods for intracellular recording and light stimulation have been described elsewhere⁴.

Figure 1*a* shows the simultaneously recorded responses from an L-type horizontal cell and a centre-hyperpolarising bipolar

cell, identified according to criteria for turtle retina defined previously^{2,5}. The stimulating light, a spot of 200 μm radius, was centred midway between the electrodes, which were about 100 μm apart. When hyperpolarising current was passed through the horizontal cell membrane, a sustained depolarisation was recorded from the centre-hyperpolarising bipolar cells (Fig. 1*b*(1)). Increasing the current induced a larger response and reduced latency (Fig. 1*b*(2)). In this cell, the lowest detectable response was evoked by injecting $1.7 \times 10^{-9} \text{ A}$, which produced an estimated -1.7 to -3.4 mV change of horizontal cell membrane potential (assuming a horizontal cell input resistance of $1\text{--}2 \text{ M}\Omega$). Similar results were obtained during illumination (Fig. 1*c*), and the bipolar cell response to current injection disappeared when the electrode was withdrawn from the horizontal cell. On the other hand, centre-depolarising bipolar cells are hyperpolarised or depolarised, respectively, by passing hyperpolarising or depolarising currents through the horizontal cell membrane (Fig. 1*e*, 1 and 2).

Thus, in contrast to the conclusions of Piccolino and Gerschenfeld³, our data show that horizontal cell activity influences the membrane potential of bipolar cells in a way qualitatively consistent with the previously proposed role of horizontal cells in mediating surround effects. This result might be expected from published anatomical and physiological evidence—electron microscopy shows that horizontal cells are presynaptic to bipolar cells at presumed chemical synapses of conventional type^{1,6} while an indirect input to bipolar cells is provided by horizontal cell feedback onto cones⁷. Although the present results do not give information on the circuitry underlying the bipolar cell responses to horizontal cell stimulation, it seems reasonable to assume that both the direct and feedback pathways are involved. The participation of additional retinal elements in mediating the bipolar cell surround cannot be excluded.

P. L. MARCHIAFAVA

Laboratorio di Neurofisiologia del CNR
Via S. Zeno, 51
56100-Pisa, Italy

Received 27 June; accepted 24 July 1978.

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Bilayers containing gangliosides develop channels when exposed to cholera toxin

CHOLERA toxin, a protein of molecular weight 84,000, comprising five or six B and one A subunits, produces massive diarrhoea in man and other mammals by altering the transport of salt and water across the intestinal epithelium¹⁻⁴. These effects are mediated by a sequence of events leading to an increase in the concentration of cyclic AMP in the epithelial cells. The sequence involves binding of the toxin through its B subunits to specific receptors which seem to be the monosialoganglioside galactosyl-*N*-acetyl-galactosaminyl-(*N*-acetyl-neuraminyl)-galactosyl-glucosyl-ceramide (GM_1), and subsequent activation of adenyl cyclase⁵⁻⁷. We report here experiments which show that cholera toxin reacts with lipid bilayers containing GM_1 to form channels which allow ions present in the aqueous solution to traverse the membrane.

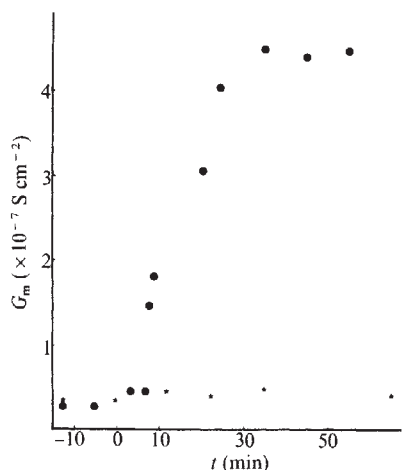


Fig. 1 Time course of development of membrane conductance (G_m) of a bilayer containing 3% G_{M1} in GMO (●) and one containing 3% G_{M2} in GMO (★) after addition at time $t = 0$ of 10 ng ml^{-1} (●) or 15 ng ml^{-1} (★) cholera toxin to one chamber. GMO concentration was 12 mg ml^{-1} .

Bilayers were formed from a mixture of glycerolmonooleate (GMO) (Nu-Chek) and G_{M1} or G_{M2} (*N*-acetylgalactosaminyl-*N*-acetylneuraminy)galactosyl-glucosyl-ceramide (Supelco) in the molar ratios indicated. The lipid mixture was dissolved in chloroform-methanol (2:1, v:v) and spread on the water-air interfaces of the bathing solutions. After allowing the solvents to evaporate (approximately 30 min), $2 \mu\text{l}$ of decane were added to each of the interfaces, a procedure which increased the stability of the membranes. The bilayers were then formed by the technique first described by Montal and Mueller⁸ and described in detail by Hall and Latorre⁹. The area of black film could not be determined accurately by visual means (the diameter of the holes used in these studies was 0.36 mm). Therefore, an estimate was made from the measured capacitance of the films (690 – 740 pF) and the value of the specific capacitance for this type of membrane formed in the absence of decane at the interfaces, by pretreating the hole with a 2% solution of squalene in pentane ($0.84 \pm 0.02 \mu\text{F cm}^{-2}$). The aqueous solutions present in the 1.5-ml chambers contained initially 100 mM NaCl buffered to pH 7.0 with 5 mM Na-phosphate buffer. The electrical characteristics were determined using a pair of Ag-AgCl electrodes to inject and measure current, and a pair of calomel electrodes to measure zero-current membrane potentials. When appropriate, a portion of a freshly diluted solution of cholera toxin (Schwarz-Mann) was added to one of the chambers.

Figure 1 shows the results of experiments in which cholera toxin was added to the aqueous solution bathing one side of GMO bilayers containing either G_{M1} or G_{M2} . An abrupt and substantial increase in membrane conductance was observed in the former but not in the latter case. GMO membranes did not respond to cholera toxin addition (not shown). Apparently, a

specific interaction between G_{M1} and cholera toxin is necessary to produce an increase in membrane conductance. This point was substantiated by experiments in which cholera toxin was mixed with excess G_{M1} and then added to the aqueous solution bathing one side of a bilayer containing G_{M1} . No increase in membrane conductance was observed. Subsequent addition of cholera toxin not previously mixed with G_{M1} to the opposite side of the membrane produced the usual conductance increase.

Examination of records of membrane current at constant membrane voltage and higher time resolution reveals the presence of current fluctuations (Fig. 2). These records are consistent with a picture in which ion-conducting channels with

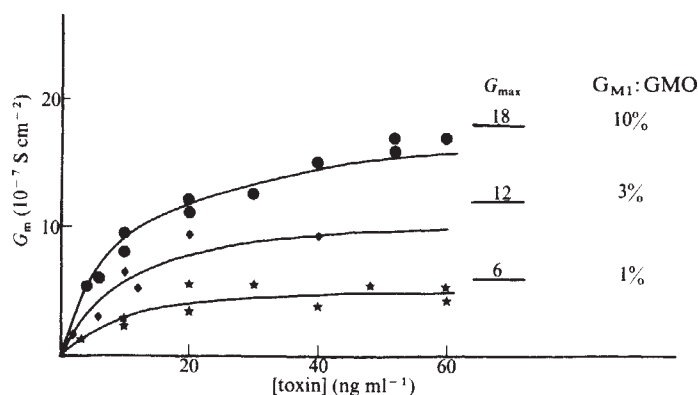


Fig. 3 Dependence of the zero-current, zero-voltage steady-state conductance (G_m) on the concentration of cholera toxin at different molar ratios of G_{M1} in GMO (12 mg ml^{-1}). The points for 1% and 10% were obtained in three different membranes, each of which was exposed to at least three different concentrations of toxin. Those for 3% are all from different membranes, averaged values. The lines were drawn according to the equation in the text, with $K_m = 1.2 \times 10^{-10} \text{ M}$ and G_{max} values as indicated.

a conductance of the order of 20 – 40 pS cm^{-2} randomly open and close during the experiment. The ionic selectivity of the channels was evaluated by measuring the zero-current membrane potential in the presence of known differences in the concentration of Na, K and Cl in the two aqueous bathing solutions. The results indicated that the channels are twice as permeable to the monovalent cations as to chloride, with practically no selectivity for Na over K.

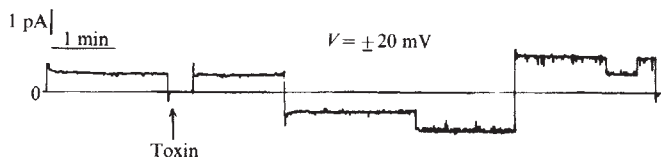
Figure 3 shows the dependence of membrane conductance on cholera toxin concentration at three different mol fractions of G_{M1} in the membrane-forming solution. The bilayer conductance levelled off as the toxin concentration in the aqueous phases was increased, and so the data were fitted using a saturating type of relation between membrane conductance and toxin concentration:

$$G_m = \frac{G_{\text{max}}[\text{toxin}]}{K_{1/2} + [\text{toxin}]}$$

where G_m is the steady-state membrane conductance, G_{max} is the maximum membrane conductance at high toxin concentration, $[\text{toxin}]$ is the concentration of cholera toxin in the aqueous phase and $K_{1/2}$ is the concentration of toxin which produced half the maximum conductance increase. The value of $K_{1/2}$ required to fit the data at the three concentrations of G_{M1} used is $1.2 \times 10^{-10} \text{ M}$, similar to the value found by Cuatrecasas for the interaction of cholera toxin with fat cell membranes⁵.

These data support a picture in which cholera toxin reacts with the sugar residues in the G_{M1} molecules in the membrane surface to produce a complex that forms ion-conducting channels. We are now investigating the size and kinetic properties of the single channels and the role of the different subunits of cholera toxin in

Fig. 2 Recorder trace showing the development of membrane conductance and current fluctuations for a GMO membrane containing 1% G_{M1} . GMO concentration, 12 mg ml^{-1} . Cholera toxin was added to a final concentration of 3 ng ml^{-1} . The jump in current shown was the only one, although at later times, current fluctuations of smaller values were also observed.



the process of channel formation. These experiments may help to clarify the mechanism by which cholera toxin, and perhaps other agonists, activate adenyl cyclase.

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M. T. TOSTESON
D. C. TOSTESON

Department of Physiology,
Harvard Medical School,
25 Shattuck Street,
Boston, Massachusetts 02115

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Human urinary kallikrein converts inactive to active renin and is a possible physiological activator of renin

WE have found that human urinary kallikrein converts inactive plasma renin *in vitro* to an enzyme with renin-like activity. This observation is interesting because renal kallikrein, the presumed precursor of urinary kallikrein¹, is thought to be situated in or on cells of the distal convoluted tubule, including the macula densa cells¹⁻³, which are adjacent to the storage site of renin, the juxtaglomerular cells⁴. Also, human urinary kallikrein is a trypsin-like enzyme of highly restricted specificity⁵, and although trypsin is capable of activating inactive renin⁶, we have found that kallikrein is at least 10 times more active. Therefore it seems possible that urinary kallikrein is the *in vivo* converting enzyme of prorenin and may activate the renin-angiotensin-aldosterone hormonal system, as it participates in sodium and potassium homeostasis⁴ and in the blood pressure maintenance of a majority of hypertensive patients⁷.

Inactive plasma renin can be converted *in vitro* to active renin by trypsin⁶, by exposure to cold (cryoactivation)^{8,9}, or by dialysis to pH 3.3 with subsequent restoration of neutral pH (ref. 10). We have shown that in plasma the major portion of the increase in renin following dialysis to pH 3.3 occurs at alkaline pH (refs 11, 12). Like cryoactivation, the alkaline phase of 'acid' activation seems to involve an unidentified endogenous neutral serine protease^{11,12}. As acidification of plasma destroys endogenous protease inhibitors^{13,14}, and as activation of inactive renin by endogenous proteases following acidification proceeds very slowly, we were able to use acidified plasma to test the potency of small amounts of highly purified urinary kallikrein¹⁵ on the conversion of inactive to active renin.

Pooled human plasma (50 ml, in presence of EDTA) with renin activity¹⁶ of 1.5 ng angiotensin I ml⁻¹ h⁻¹ was dialysed to pH 3.3 for 24 h at 4 °C. Two 1-ml samples were further dialysed from pH 3.3 to 7.4 at 4 °C for 24 h after which renin averaged 8.5 ng ml⁻¹ h⁻¹ (Fig. 1). Plasma renin activity was 4.5 ng ml⁻¹ h⁻¹ in the remainder of the pool which was adjusted to pH 7.4 and there was no further increase during 1 h incubation at 25 °C and pH 7.4. When urinary kallikrein or trypsin was added to this pool for 1 h at 25 °C, a 66% increase in renin activity was observed with urinary kallikrein 2 × 10⁻⁷ M but only a 39% increase with trypsin 2 × 10⁻⁶ M (Fig. 1). Maximum activation (that is, to 8.3 ng ml⁻¹ h⁻¹) was achieved by urinary kallikrein 4 × 10⁻⁷ M, whereas trypsin 8 × 10⁻⁶ M did not quite achieve maximum activation.

In another study urinary kallikrein at 0, 2.0 and 3.1 × 10⁻⁶ M was added to untreated plasma and incubated for 2 d at -4 °C. In the absence of urinary kallikrein there was a small increase in renin activity due to cryoactivation⁹. However, urinary kallikrein caused a concentration-dependent increase in the rate of cryoactivation (Fig. 2), thus demonstrating that activation of inactive renin by kallikrein does not depend on previous acidification.

Although inactive renin is present in the plasma of most anephric patients¹⁷, the data in Fig. 3 demonstrate that it is also of renal origin. Active and inactive plasma renin were measured in simultaneously collected renal vein and inferior vena caval (IVC) samples from a patient with renal artery stenosis. Active and inactive renin levels were the same in the IVC and right renal vein, but there were marked increments in both forms of renin in renal venous plasma from the left side, thus demonstrating renal secretion of both active and inactive renin.

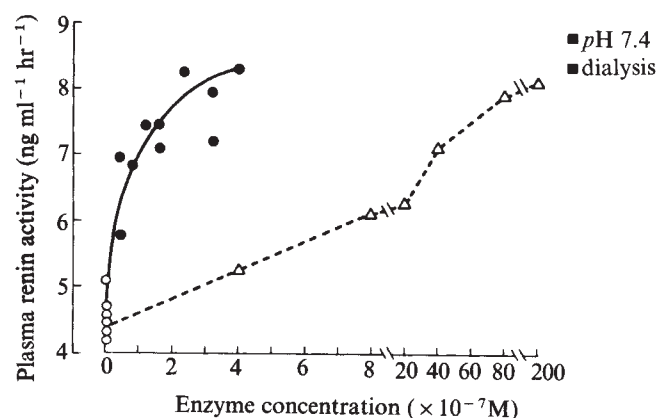


Fig. 1 Activation of inactive renin in previously acidified plasma by urinary kallikrein, trypsin or endogenous serine proteases. Pooled human plasma (50 ml; EDTA) was dialysed to pH 3.3 for 24 h at 4 °C (0.05 M glycine-HCl, 0.1 M NaCl, 3 mM Na₂EDTA). This destroys endogenous protease inhibitors^{13,14} and activates about one-third of inactive plasma renin¹¹. Three methods of activation of the remaining inactive renin were tested. ■, Two 1-ml samples were further dialysed to pH 7.4 for 24 h at 4 °C (0.10 M sodium phosphate, 0.05 M NaCl, 3 mM Na₂EDTA) during which time endogenous proteases slowly activated inactive renin. The remainder (48 ml) was adjusted to pH 7.4 at 0 °C with 5 ml 4 M Tris, pH 7.4, and 0.5 ml 2 M NaOH. ●, Pure urinary kallikrein¹⁵ (specific activity 1094 µg equivalents bradykinin min⁻¹ mg⁻¹ at pH 8.5 and 37 °C); or △, trypsin (Boehringer Mannheim, specific activity 33 U mg⁻¹); or ○, distilled water, were added to 0.5 ml samples which were then incubated at 25 °C for 1 h. Activation was stopped by adjustment to pH 5.7 and addition of phenylmethylsulphonyl fluoride (PMSF) 1.5 mmol l⁻¹. Renin activity was then measured by incubation at pH 5.7 and 37 °C (ref. 16) after addition of partially purified human renin substrate¹¹. Results for urinary kallikrein were from three separate experiments.

Stop-flow² and histological studies³ have suggested that renal kallikrein is located in the cells lining the distal convoluted tubule extending from the juxtaglomerular apparatus (where renin is synthesised) to the collecting duct. Its activity has been associated with the plasma membrane and/or endoplasmic reticulum^{1,18} of cells from the renal cortex, although lysosomal fractions have also been implicated¹⁹. Thus, kallikrein may be present in the renin-secreting cells of the juxtaglomerular apparatus. Urinary kallikrein is reported to have a more restricted specificity than trypsin⁵ and is often catalytically less efficient^{20,21}. Its greater potency for activating inactive renin would therefore seem to favour specific interaction. This observation, together with its location in the kidney, suggests that kallikrein might be a prorenin converting enzyme. However, other mechanisms may be involved, as inhibitors of sulphhydryl-dependent enzymes have been reported to block conversion of

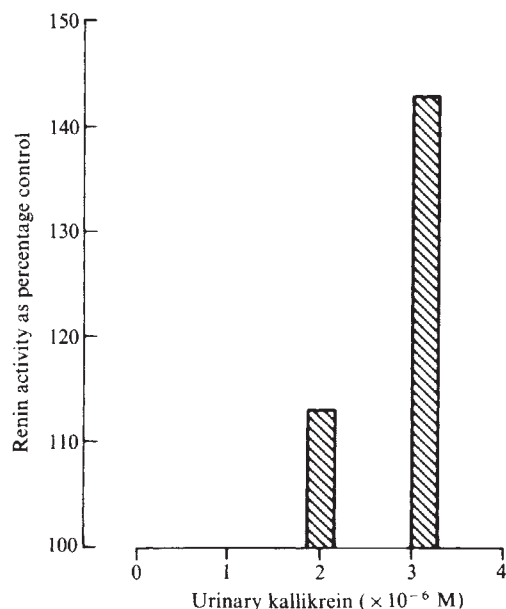


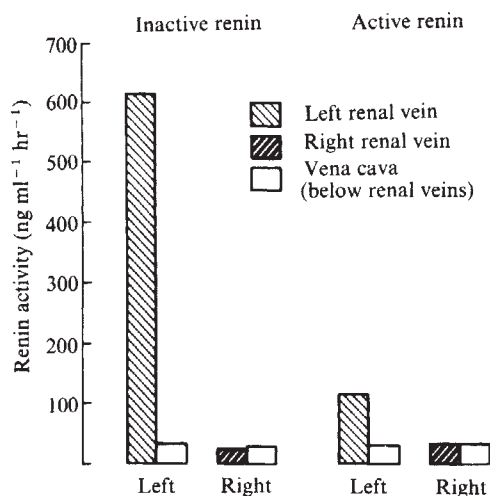
Fig. 2 Potentiating effect of urinary kallikrein on the rate of cryoactivation of inactive plasma renin: 100 or 200 μ l (2×10^{-6} and 3.1×10^{-6} M) purified urinary kallikrein or distilled water was added to 0.3 ml plasma (EDTA) which was then incubated at -4°C for 38 h (96 h are required for cryoactivation to reach a plateau). Renin was then assayed at pH 5.7 and 37°C in the presence of 1.5 mM PMSF. Renin activity, measured after cryoactivation in the presence of urinary kallikrein, is expressed as a percentage of that measured after 38 h cryoactivation alone.

large to small molecular weight hog renal renin with a resultant small change in activity²².

If kallikrein activates inactive renin *in vivo*, this would be the second link between the kallikrein and renin systems, as angiotensin-converting enzyme converts angiotensin I to angiotensin II and also hydrolyses bradykinin to inactive products²³.

So far, urinary kallikrein is the most potent activator of inactive renin yet described and may be strategically located to

Fig. 3 Unilateral renal secretion of active and inactive renin from a hypertensive patient with left renal artery stenosis. Blood specimens were collected from the IVC simultaneously with each renal vein blood collection. Inactive plasma renin was measured as the difference between endogenous renin activity and the renin activity following activation by trypsin (1 mg per ml plasma at -4°C for 1 h). There was no difference in active or inactive renin between the IVC and right renal vein, whereas a marked increment in both renins was observed from the left side, thus demonstrating the renal origin of both active and inactive renin.



participate in renin biosynthesis. Whether the enzyme is part of a biological control system for the release of renin and for the accomplishment of sodium, potassium and blood pressure homeostasis, remains to be established.

JEAN E. SEALEY
STEVEN A. ATLAS
JOHN H. LARAGH

Cardiovascular Center,
New York Hospital-Cornell University Medical Center,
New York, New York 10021

NARENDRA B. OZA
JAMES W. RYAN

Department of Medicine,
University of Miami School of Medicine,
Miami, Florida 33152

Received 15 May; accepted 28 July 1978.

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Specific and irreversible block of the myotropic action of angiotensin II

COMPETITIVE inhibitors of the octapeptide hormone angiotensin II (AT), Asp-Arg-Val-Tyr-Val-His-Pro-Phe, have been synthesised by several groups¹. These inhibitors, like the hormone, are quickly degraded, and no irreversible or even long-acting specific inhibitors have been available. Paiva *et al.*² have attempted to produce such inhibitors using affinity labelling analogues, but with poor results. We report here a specific and irreversible inhibition of the response of rabbit aorta strips to AT by a photolabile analogue of AT.

We previously described the synthesis³ and the biological activities⁴ of the AT analogue Sar-Arg-Val-Tyr-His-Pro-(4'-azido)Phe (= N₃-AT) in the absence of ultraviolet radiation. Also, (4'-azido)Phe has been successfully used as a photoaffinity label in studies on chymotrypsin⁵ and ribosomes⁶. In the present study rabbit aorta strips were suspended in Krebs's solution according to the method of Rioux *et al.*⁷. Photolysis was carried out with four parabolic mercury vapour lamps (Westinghouse JC PAR-38) equipped with black light filters surrounding a tissue bath. The light was further filtered through pyrex glass and

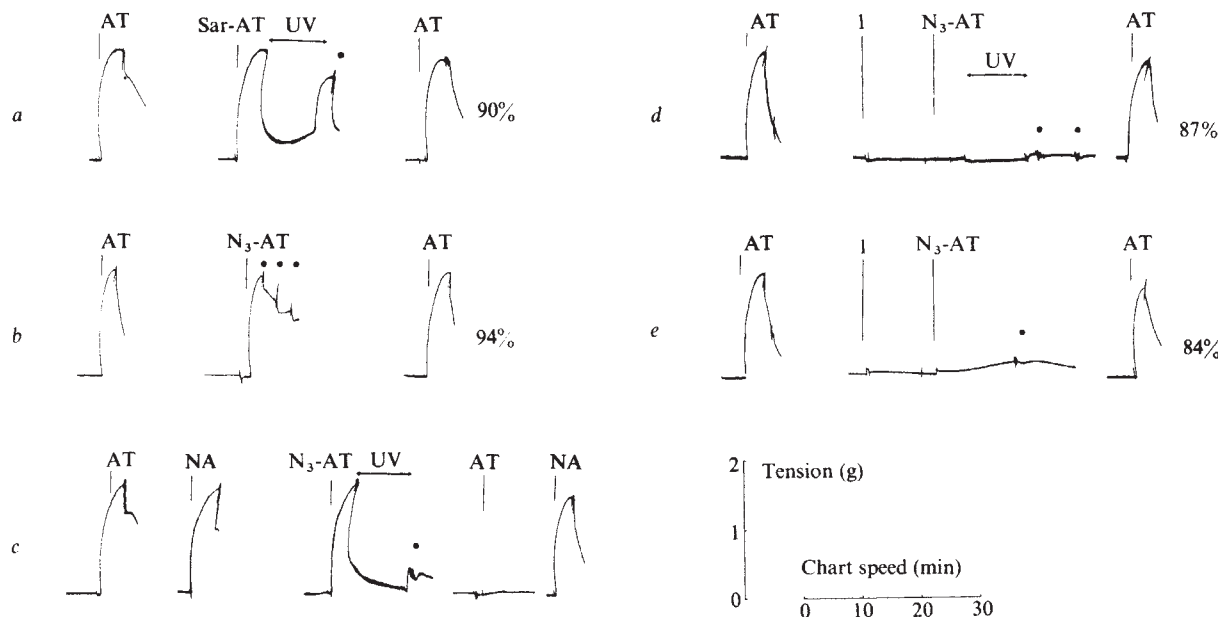


Fig. 1 Contraction of rabbit aorta strips in various conditions. The interval between injections of angiotensin II (AT), 200 ng ml⁻¹, (Sar¹)-angiotensin II (Sar-AT), 200 ng ml⁻¹, or its photolabelling analogue (Sar¹(4'-azido)Phe⁸)-angiotensin II (N₃-AT), 200 ng ml⁻¹, was 90 min. Noradrenaline (NA), 50 ng ml⁻¹, was injected 30 min after AT, and (Leu⁸)-angiotensin II (I), 5,000 ng ml⁻¹, was added 15 min before the agonist. All tests have been carried out under total exclusion of UV radiation except when indicated. Dots indicate washing of the tissues. Contractions of the rabbit aorta strips suspended in tissue baths were recorded on a Grass polygraph with isometric force transducers. All other experimental details are as described by Rioux *et al.*⁷.

water so that almost only the 365-nm band of the mercury spectrum reached the tissue.

When tissues contracted by (Sar¹)-AT were irradiated, they relaxed immediately (Fig. 1a), analogous to the results reported by Furchgott *et al.*⁸. If the irradiation is discontinued the tissues regain their initial degree of contraction and are normally responsive to further applications of stimulants. In the dark, N₃-AT acts as a reversible agonist of the AT receptor (Fig. 1b); it has the same intrinsic activity as (Sar¹)-AT and a relative affinity of 31%. If the tissues contracted by N₃-AT (Fig. 1c) are irradiated, the strips relax as they do under the influence of light, but they do not recontract when the light is removed. They also lose their responsiveness to AT but not to noradrenaline (Fig. 1c), histamine, bradykinin, 5-hydroxytryptamine or potassium (not shown), agents acting on receptors different from those of AT. Further applications of AT during the following 2–6 h do not induce any significant rise in tension. If the same experiment is carried out in the presence of the competitive inhibitor (Leu⁸)-AT at saturating concentrations, the tissues recover their responsiveness to AT (Fig. 1d). The slight decrease in response observed is due to some desensitisation by the inhibitor and it also occurs in the same experiment without irradiation (Fig. 1e).

In some preparations (one out of five), in which the desensitisation of the tissues to AT achieved with photolysis of N₃-AT was less than 80%, the treatment was repeated and resulted in an almost complete inactivation.

Two possible reactions of the tissue were expected with a specific covalent block by an agonist. (1) The tissue stays contracted due to a continuous stimulation of the response by a stable hormone–receptor complex. This phenomenon has been reported with gastrin⁹. (2) Covalent attachment of the hormone to the receptor induces a permanent loss of hormone response, because stimulation occurs only when the receptor changes from 'bound' to 'unbound' or the reverse. If the hormone is irreversibly attached this dynamic process cannot occur again. We therefore conclude that, under photolysis, N₃-AT covalently and specifically blocks the receptor for AT. Studies using radioactive analogues of N₃-AT are now in progress in an attempt to isolate the AT receptor from arterial smooth muscle.

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EMANUEL H. F. ESCHER*
T. MAI DUNG NGUYEN
GAËTAN GUILLEMETTE
DOMENICO C. REGOLI

Department of Physiology and Pharmacology,
Faculty of Medicine,
University of Sherbrooke,
Sherbrooke, Québec, Canada J1H 5N4

Received 1 May; accepted 30 June 1978.

* To whom correspondence should be addressed.

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Evidence for an essential DNA component in the Scrapie agent

ONE of the enigmas of microbiology has been the nature of the aetiological agent causing scrapie disease in sheep and goats. The unusual resistance of infectivity to heat, formaldehyde, nucleases and UV irradiation has stimulated many hypotheses, including speculation that the scrapie agent may lack nucleic acid¹. This stability to physical and chemical inactivation has been due in part to the intimate association of the agent with the cell membrane^{2,3}, making it impossible to obtain soluble fractions of brain tissue containing high titres of infectivity. A

hamster model of scrapie disease has been developed^{4,5} that has shorter incubation periods and higher brain titres of infectivity than any other animal model. Studies on the subcellular distribution of scrapie agent in hamster brain have shown that 8–9% of the total infectivity can be found in membrane-free high-speed supernatant (HSS) fractions⁶. Infectivity in the HSS exists as small heterogeneous complexes which are resistant to detectable enzymatic degradation, but are more sensitive to inactivation by heat and UV irradiation than are cruder brain preparations⁷. We report here further partitioning of scrapie infectivity in the HSS using two methods of separation, hydroxyapatite chromatography and polyacrylamide gel electrophoresis, both of which result in isolation of a fraction of scrapie infectivity that is inactivated by deoxyribonuclease (DNase). We therefore conclude that the scrapie agent contains an essential DNA component.

Hydroxyapatite chromatography was performed as reported previously⁷. Briefly, brain homogenates in 12 mM sodium phosphate (pH 7.2) are centrifuged at 100,000g for 18 h in a Beckman 42.1 rotor and the supernatant fractions applied to a hydroxyapatite column at room temperature. Scrapie infectivity eluted in the 0.48 M sodium phosphate wash, and representing 1% of the total input infectivity to the column, was diluted 1:4 with buffer (100 mM Tris, 0.15 M NaCl, 12 mM sodium phosphate, pH 7.2), then treated with DNase I (Worthington), RNase A (Worthington) or proteinase K (Boehringer-Mannheim) at concentrations of 100 µg ml⁻¹, 50 µg ml⁻¹ or 62 µg ml⁻¹, respectively. Magnesium chloride (20 mM) was added to the tube containing DNase and also to the control tube which received no enzyme treatment. All preparations were then incubated in a water bath at 37 °C for 60 min. To ensure that the enzymes used were biochemically active in the conditions stated, they were individually tested on radioisotope-labelled substrates which were added directly to the scrapie sample to be treated. Scrapie infectivity was measured by intracerebral inoculation of weanling hamsters and endpoints calculated 16 weeks after injection using the method of Spearman and Kärber⁸. As can be seen in Table 1, only treatment with DNase resulted in substantial reduction of infectivity.

Because the above experiment indicated that infectivity was resistant to proteolytic digestion, a second sample of HSS was digested with 100 µg ml⁻¹ proteinase K before adsorption to hydroxyapatite. The hydroxyapatite eluate from the proteinase K-digested HSS contained a 10-fold higher titre than that of the undigested HSS, and a greater proportion of the total scrapie infectivity was inactivated with DNase (Table 1).

In support of the above findings, we have since succeeded in obtaining a purified preparation of scrapie agent of higher titre using ammonium sulphate precipitation of the HSS and separation of the resuspended precipitate by polyacrylamide gel electrophoresis⁹. Enzyme treatment of scrapie infectivity from these gels (Table 1), in conditions similar to those described for the treatment of the hydroxyapatite eluates, also resulted in a significant decrease in scrapie activity after exposure to DNase (100 µg ml⁻¹), but not to RNase A (100 µg ml⁻¹) or proteinase K (100 µg ml⁻¹).

These results show that as the heterogeneous infectious complexes in the HSS are separated, either by hydroxyapatite

chromatography or polyacrylamide gel electrophoresis, fractions of infectivity can be detected which are sensitive to treatment with DNase. It is, therefore, reasonable to conclude that the scrapie agent contains an essential DNA component. This finding is compatible with other studies^{3,10,11} which have detected low-molecular-weight DNA sequences in scrapie-infected brain, but which have been of questionable significance because of the inability to relate these nucleic acids to infectivity. However, the exact nature, size and possible association of this scrapie-specific DNA with other macromolecules requires further study.

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R. F. MARSH

Department of Veterinary Science,
University of Wisconsin,

TERRY G. MALONE
J. S. SEMANCIK

Department of Plant Pathology,
University of California-Riverside,
Riverside, California 92502

WAYNE D. LANCASTER*
R. P. HANSON

Department of Veterinary Science,
University of Wisconsin,
Madison, Wisconsin 53706

Received 1 May; accepted 30 June 1978.

* Present address: Division of Otolaryngology, Case Western Reserve University, Cleveland, Ohio 44106.

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Highly conserved DNA of Ti plasmids overlaps T-DNA maintained in plant tumours

LARGE tumour-inducing (Ti) plasmids in *Agrobacterium tumefaciens*¹ are responsible for crown gall disease in dicotyledonous plants^{2,3}. Plant tumour cells cultivated *in vitro* in the absence of the inciting bacterium maintain multiple copies of part of the Ti plasmid^{4,5}. The experiments presented here show that part of the Ti plasmid maintained in tumour cells (T-DNA) is highly conserved among diverse Ti plasmids.

Ti plasmids from a variety of natural isolates of *A. tumefaciens* exhibit only partial DNA homology^{6,7}. Their fingerprints, produced by cleavage of plasmid DNA with restriction endonucleases, are markedly different^{8–11}. Ti-plasmid-borne genetic traits (octopine and nopaline metabolism)^{2,3,12–15} suggest that Ti plasmids are of three types, and physical studies confirm this^{6–11}. Schell and his collaborators¹⁶ have suggested that since common tumour-inducing functions are a feature of every Ti plasmid, a region of homologous DNA should be detectable in all types. We have mapped the plasmid pTi-B6-806 (ref. 17) and located the T-DNA, the region of this plasmid that is maintained in E9 tobacco tumour (Fig. 1)^{4,18}. We report

Table 1 Enzyme treatment of scrapie infectivity separated by hydroxyapatite chromatography (HA) or polyacrylamide gel electrophoresis (PAGE)

Enzyme treatment	HA (undigested)	HA (predigested)	PAGE
None	4.0*	5.0	7.5
RNase A (50–100 µg ml ⁻¹)	4.2	5.0	7.8
Proteinase K (62–100 µg ml ⁻¹)	4.0	ND†	7.5
DNase I (100 µg ml ⁻¹)	2.5	2.8	4.2

*log₁₀ LD₅₀ ml⁻¹.

†Not determined.

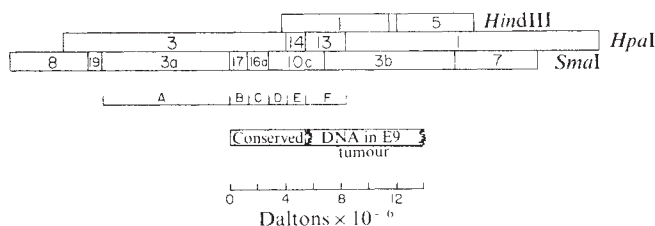


Fig. 1 Map of *Sma*I, *Hpa*I and *Hind*III restriction endonuclease cleavage sites in part of pTi-B6-806. Evidence for the map order is given in detail elsewhere^{17,18}. Certain fragments of pTi-B6-806 used in this study were designated A-F as indicated above for clarity. Evidence for the boundaries of the DNA detected in E9 tumour (T-DNA) is presented elsewhere^{4,5,18}.

here DNA hybridisation studies that test this region of pTi-B6-806 for homology with six representative Ti plasmids.

A map of the sector of pTi-B6-806 that is relevant to this study is presented in Fig. 1. For the DNA hybridisation studies described below, pTi-B6-806 DNA fragments to be used as probes were isolated after preparative agarose gel electrophoresis of plasmid DNA digests⁴. Fragments were labelled by the 'nick translation' reaction¹⁹.

Figure 2 presents *Sma*I cleavage patterns of the Ti plasmids examined here. Nopaline-type plasmids are diverse, and are

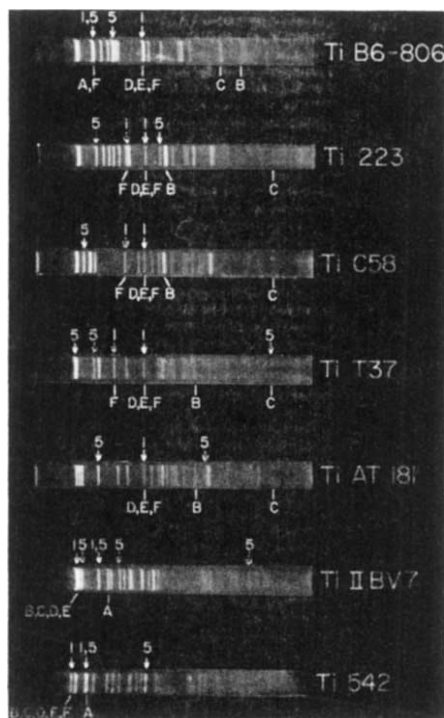


Fig. 2 *Sma*I cleavage patterns of diverse Ti plasmids. Descriptions and sources of bacterial strains are detailed elsewhere¹⁰. Plasmid DNA (2.5 µg) isolated as described previously¹⁰ was adjusted to 0.02 M Tris, pH 7.5, 15 mM KCl, 6 mM MgCl₂ and treated with sufficient *Sma*I to give a complete digest in 2 h at 30°C. *Sma*I was purified by the procedure of Lyle Brown, Oregon State University (private communication). Digests were subjected to horizontal slab gel electrophoresis¹⁰ and bands were visualised by staining for 20 min with ethidium bromide (10 µg/ml) and illuminating on a Blak-Ray Transilluminator (UV Products Inc.). Arrows above each digest indicate the positions of fragments that hybridised strongly (solid arrows) or weakly (dotted arrows) with *Hind*III fragments 1 and 5; the probe is indicated by the number above each arrow. The letters beneath each digest indicate the positions of fragments that hybridised with probes A-F, as defined in Fig. 1.

represented by pTi-223, pTi-C58, pTi-T37, pTi-AT181 and pTi-IIBV7. The only known null-type plasmid is pTi-542⁹. Octopine-type plasmids are highly conserved⁶⁻¹⁰ and are represented by pTi-B6-806, the homologous DNA control. On the basis of DNA homology measurements^{6,7} pTi-IIBV7 and pTi-542 are more closely related to the octopine-type plasmid than are any of the other plasmids studied here.

Digest fragments of Ti plasmids (Fig. 2) were transferred from the agarose gel to sheets of cellulose nitrate (Schleicher and Schuell, B6 membrane) by a modification²⁰ of the Southern blotting procedure²¹. Transfers were cut into longitudinal strips for hybridisation with various labelled DNA fragments.

*Hind*III fragment 1 labelled DNA hybridised with most of the heterologous plasmids extensively, as shown in the autoradiograms of Fig. 3 (data not shown for pTi-542). In four of the heterologous plasmid digests, the labelled DNA bound almost exclusively to a fragment of approximately the same electrophoretic mobility as *Sma*I fragment 10c of pTi-B6-806

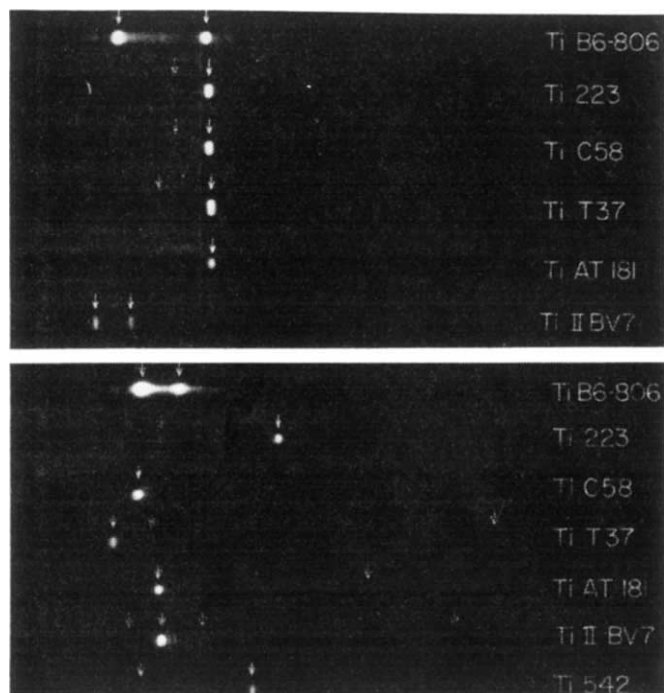


Fig. 3 Autoradiograms of hybrids formed with labelled *Hind*III fragments 1 and 5 as probes. DNA fragments used as probes were labelled to specific activities of $1-50 \times 10^6$ c.p.m. per µg by incorporation of [α -³²P]thymidine triphosphate (90-150 Ci mmol⁻¹) or [α -³²P]deoxycytidine triphosphate (1,500 Ci mmol⁻¹) (NEN) using the nick translation reaction¹⁹. Cellulose nitrate transfer strips prepared from the gel of Fig. 2 or similar gels were allowed to hybridise with labelled DNA fragments (10^5 c.p.m. per ml in $2.5 \times$ SSC at 63°C for 18 h) as described elsewhere (SSC is 0.15 M NaCl, 0.015 M trisodium citrate). The solid and dotted arrows above the bands indicate the intensity of hybridisation of the probes, and correspond to solid and dotted arrows in Fig. 2. Upper set of autoradiograms, *Hind*III fragment 1 probe. Lower set, *Hind*III fragment 5 probe.

(solid arrows marked 1 in Fig. 2). In the remaining two cases, pTi-IIBV7 and pTi-542, two fragments of higher molecular weight bound the labelled probe less efficiently. As expected from the map position of *Hind*III fragment 1 (Fig. 1), this probe hybridised strongly with pTi-B6-806 *Sma*I digest bands 3 and 10.

Thermal dissociation profiles of the DNA/DNA duplexes formed with *Hind*III fragment 1 probe are presented in Fig. 4. The T_m (midpoint of thermal dissociation) of homologous duplexes, 86°C, is 3-5°C higher than that of the heterodu-

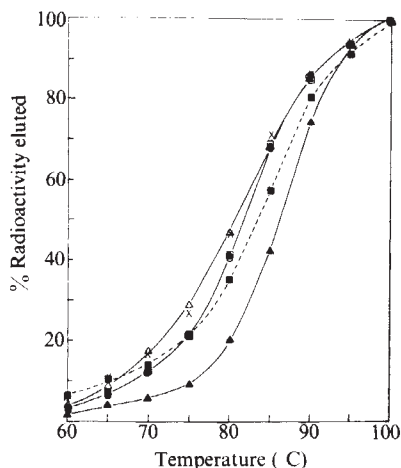


Fig. 4 Thermal dissociation profiles of *Hind*III fragment 1 duplexes. The autoradiogram of the upper portion of Fig. 3 was used as a guide to locate bands of DNA duplexes corresponding to the solid arrows. The cellulose nitrate segment bearing these duplexes was excised and placed in a 10×75 mm test tube. The segment was eluted with 1 ml aliquots of 1×SSC at 22°, 60°, 65°C and so on and radioactivity eluted at each temperature was assayed. Radioactivity eluted at 22°C and that remaining on the cellulose nitrate segment at the end of the melt were negligible. Total radioactivity eluted was 1,500–2,000 c.p.m. in all cases. ▲, pTi-B6-806 ($T_m = 86^\circ\text{C}$); △, pTi-IIBV7 ($T_m = 81^\circ\text{C}$); □, pTi-223 ($T_m = 82^\circ\text{C}$); ●, pTi-C58 ($T_m = 82^\circ\text{C}$); ○, pTi-T37 ($T_m = 82^\circ\text{C}$); ×, pTi-AT181 ($T_m = 82^\circ\text{C}$); ■, pTi-542 ($T_m = 83.5^\circ\text{C}$).

plexes, indicative of 3–5% base-pair-mismatching in the latter²⁴.

Labelled *Hind*III fragment 5 of pTi-B6-806 hybridised less extensively to all heterologous plasmids than to the homologous one (Fig. 3). The arrows marked 5 in Fig. 2 indicate the fragments that bound this labelled probe well (solid arrows) or poorly (dotted arrows). We have not examined in detail which part of *Hind*III fragment 5 is conserved in the heterologous Ti plasmids.

We obtained a more detailed picture of the extent of the conserved DNA region within and to the left of *Hind*III fragment 1 by using as labelled probes *Sma*I and *Hpa*I digest fragments of pTi-B6-806. The 42 autoradiograms from these experiments are not shown here, but the results are summarised in Fig. 2. The labelled probes are designated by serial letters A–F, corresponding to their left-to-right map order as indicated in Fig. 1. Probe A hybridised with only two heterologous plasmids, pTi-542 and pTi-IIBV7, at positions marked A in Fig. 2. In contrast to probe A, probe F hybridised efficiently with most of the Ti plasmids, but very poorly with pTi-542 and not detectably with pTi-IIBV7 (Fig. 2).

Probes B, C, D and E hybridised with all of the Ti plasmids examined, and must therefore constitute a region of DNA common to all (Fig. 2). This common DNA region extends on the map from *Sma*I fragment 17 to *Hpa*I fragment 14, a region of 5.5×10^6 daltons or less. Probe F (*Hpa*I fragment 13) could also be considered a part of the common DNA region, for its DNA sequences are preserved on most of the Ti plasmids examined. However, the region of conserved DNA indicated in Fig. 1 does not include this imperfectly conserved fragment. The T-DNA portion of pTi-B6-806 (Fig. 1) is at least contiguous with this conserved DNA element, and since the left boundary of T-DNA is not precisely known, it is possible that it overlaps the conserved DNA region. The T-DNA of a T37 tumour line under investigation in this laboratory contains all of this conserved DNA region from pTi-T37 (ref. 22).

If there have been no gross rearrangements of DNA in these plasmids during evolution, the results presented here predict

the serial map order of the heterologous Ti plasmid fragments to be the same as that of the pTi-B6-806 probe fragments A through F, to which they hybridise (Fig. 2). We have confirmed this order by conventional mapping techniques¹⁷ for pTi-T37 (ref. 23).

It seems reasonable to predict that crown gall tumour lines incited by bacterial strains carrying the heterologous Ti plasmids studied here will contain segments of those plasmids that are proximal to the conserved DNA sequences. The results presented here show, however, that a significant portion of the T-DNA of pTi-B6-806 comprises DNA sequences not common to all Ti plasmids. The right half of *Hind*III fragment 1 is not conserved, for all of the hybridisation of this probe is ascribable to probes E and F (Figs 1 and 2), which overlap its left end. The non-conserved part of the T-DNA may code for octopine production in pTi-B6-806, a trait not shared by nopaline-type Ti plasmids, which code for nopaline production by tumours^{8,9,13,14}.

The genetic evidence of Depicker *et al.*²⁵ in the accompanying paper indicates that the common DNA sequences analysed here do indeed code for functions essential to oncogenicity. This DNA could code for functions related to the process of transformation, or for functions required in the tumour cell for maintenance of the transformed state. Transcripts of Ti plasmid DNA have been detected in crown gall tumour tissue²⁶, but it is not clear whether the common DNA region of pTi-B6-806 is transcribed. Further evidence is needed for an understanding of the role of this highly conserved DNA element in crown gall tumour induction.

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MARY-DELL CHILTON

MARTIN H. DRUMMOND

DONALD J. MERLO

DANIELA SCIACKY

Department of Microbiology and Immunology,
University of Washington, Seattle, Washington 98195

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Homologous DNA sequences in different Ti-plasmids are essential for oncogenicity

LARGE plasmids, called Ti-plasmids¹, are responsible for the transformation of dicotyledonous plants by *Agrobacterium tumefaciens*²⁻⁵. A comparison of Ti-plasmids derived from different *Agrobacterium* strains⁶⁻¹⁰ revealed two major groups of homology. These correlate with the Ti-plasmid-determined properties of octopine or nopaline utilisation by Ti-plasmid-harbouring agrobacteria^{2,5,11-13}, and octopine or nopaline synthesis by Ti-plasmid-transformed plant cells^{11,13}. It was originally suggested that octopine or nopaline synthesis in crown-gall tissues might be mediated by bacterial genes, transferred to the transformed plant cells¹⁴; this can now be restated as being mediated by Ti-plasmid genes, and is supported both by genetic data¹⁵ and by hybridisation studies¹⁶. The capacity to determine both the catabolism and the synthesis of one or the other of these or similar compounds seems to be the dominant evolutionary significance of the Ti-plasmids and one can therefore speak of 'octopine'- and 'nopaline'- Ti-plasmids. In view of the fact that the different types of Ti-plasmids only have a limited degree of DNA sequence homology⁶, but produce transformed plant cells with similar phenotypes, it was conceivable that at least part of the common DNA sequences would be directly and/or indirectly involved in oncogenicity. We report here the finding of a specific DNA segment which is highly conserved among several different *Agrobacterium tumefaciens* Ti-plasmids. We also give genetic evidence that this region is essential for oncogenicity and overlaps with the Ti-plasmid DNA found in transformed plant cells.

The Ti-plasmid of strain ACH5 was used as an example of an octopine Ti-plasmid. This strain contains only one large plasmid (molecular weight 120×10^6), which was shown by heteroduplex analysis⁸ to be completely homologous to other octopine Ti-plasmids, such as the Ti-plasmid of strain B6S3. pTi-ACH5 DNA, isolated as described previously¹³ and radioactively labelled *in vitro* by nick translation¹⁸, was hybridised with the unlabelled DNA fragments obtained by digestion of the pTi-DNA from the nopaline strains C58 and K14 with various restriction endonucleases. Strong hybridisation was observed with only one segment of both nopaline Ti-plasmids. This segment was contained in one particular fragment of an *Eco*RI digest (fragment 1, MW 9.5×10^6), as can be seen for pTi-C58 DNA in Fig. 1. The largest band of the pTi-C58 *Eco*RI restriction pattern is, in fact, a doublet. After separation of the two fragments, it was demonstrated that the dominant hybridisation with pTi-ACH5 DNA was located in the *Eco*RI-1 fragment of pTi-C58 DNA. Other nopaline Ti-plasmids (from strains 223, T37, 396) were similarly tested for homology with pTi-ACH5 and it was found that they all contained an *Eco*RI fragment of MW 9.5×10^6 that showed a strong hybridisation with pTi-ACH5. This was particularly striking, as Ti-plasmids in avirulent mutant strains derived from strain K14 (ref. 15) did not exhibit this strong hybridisation.

Reciprocal experiments were carried out by hybridising total labelled pTi-C58 DNA with unlabelled DNA fragments of the octopine plasmid pTi-ACH5. Strong hybridisation was observed with the pTi-ACH5 *Hind*III fragment 1 (8.0×10^6 daltons) as can be seen in Fig. 1. These results thus indicated that this region is common to octopine and nopaline Ti-plasmids. Note here that various other fragments of these two types of Ti-plasmids exhibited a variable degree of hybridisation, as can be seen in Fig. 1, probably indicating a lesser extent of homology rather than an experimental artefact, as it was found that the extent of these hybridisations depended on the stringency of the hybridisation conditions used. Thus, it was found that in standard hybridisation conditions (see legend to Fig. 1), up to 80% of all the fragments of the pTi-ACH5 plasmid

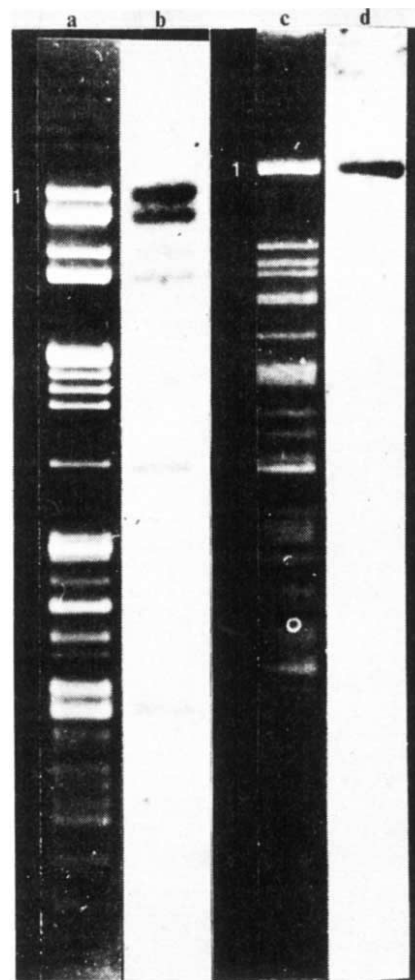
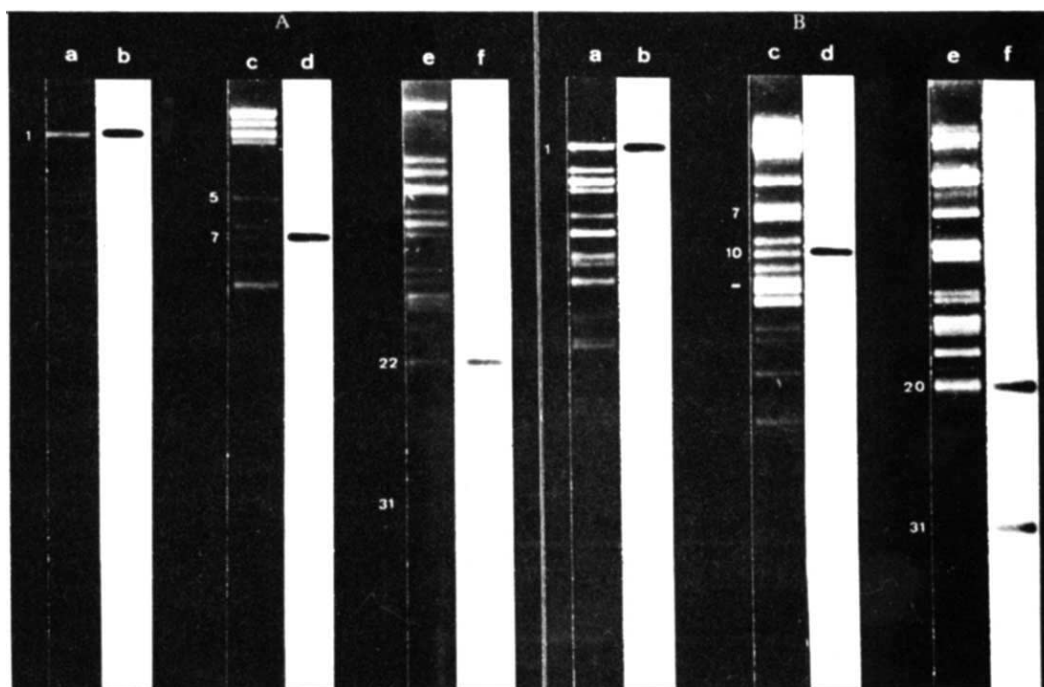


Fig. 1 Homology between octopine and nopaline Ti-plasmids. Plasmid DNA was prepared from wild-type strains ACH5 and C58 (natural isolates), as described previously¹³. The restriction enzymes *Eco*RI and *Sma*I were purified in this laboratory. The conditions for digestion were 90 mM Tris, pH 7.5, 50 mM NaCl and 5 mM MgCl₂ for *Eco*RI, 15 mM Tris, pH 8.5, 15 mM KCl and 6 mM MgCl₂ for *Sma*I and 7 mM Tris, pH 7.5, 60 mM NaCl and 7 mM MgCl₂ for *Hind*III. The digests were separated in 0.8% agarose, formed in 40 mM Tris-acetic acid, pH 8, 20 mM NaAc, 1 mM EDTA and 0.5 $\mu\text{g ml}^{-1}$ ethidium bromide, on a horizontal slab gel apparatus²⁰. Fragments of decreasing molecular weight were represented by increasing numbers; only one number was given to single bands consisting of several fragments. The λ -*Eco*RI and λ -*Hind*III fragments were used as molecular weight standards. After electrophoresis (40 V, 16 h) and photographing of the restriction pattern²¹, the DNA fragments were transferred to a nitrocellulose filter (Schleicher and Schuell no. B6) according to the Southern blotting procedure¹⁷. By nick translation, the probe DNA was labelled with ³²P to specific activities of $1\text{--}30 \times 10^6$ c.p.m. μg^{-1} , using [α -³²P]-guanosine triphosphate (Amersham, 350 Ci mmol⁻¹)¹⁸. After preincubation of the filters for 3 h at 75 °C in 2 SSC, containing 0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin (BSA) and 20 $\mu\text{g ml}^{-1}$ denatured salmon sperm DNA²², the denatured radioactive probe solution was added to the filters in a final concentration of 20 $\mu\text{g ml}^{-1}$. The filters were incubated for 24–40 h at 75 °C and were dried and exposed to X-ray film (Fuji films Rx Medical) after 4 or 5 serial washes (3 SSC, 0.5% SDS at 75 °C). The incubation temperature used in these experiments was therefore more stringent than the standard conditions used for the experiments shown in Figs 2 and 3, where the filters were incubated at 68 °C. A, pTi-ACH5 *Hind*III restriction pattern (lane a) and hybridised with ³²P-labelled pTi-C58 DNA. B, pTi-C58 *Eco*RI restriction pattern (lane c) and hybridised with labelled pTi-ACH5 DNA. The numbers indicate the fragments showing the strongest hybridisation between the two types of plasmids.

Fig. 2 Characterisation of the homologous sequence between octopine and nopaline plasmids. The restriction digests, blotting of the DNA fragments to nitrocellulose filters and hybridisations were carried out as described in the legend to Fig. 1. A, pTi-C58: *EcoRI* (a), *SmaI* (c) and *HindIII* (e) restriction patterns, transferred to nitrocellulose filters and respectively (b, d, f) hybridised with the labelled pTi-ACH5 *HindIII* fragment 1 probe. This probe (pGV0201) was in fact a hybrid plasmid constructed by insertion of pTi-ACH5 *HindIII* fragment 1 in the vector pBR322 (ref. 23). B, pGV3505 (pTi-K14): *EcoRI* (a), *SmaI* (c) and *HindIII* (e) restriction patterns, transferred to nitrocellulose filters and respectively (b, d, f) hybridised against labelled pTi-ACH5 *HindIII* fragment 1 probe.



obtained by digestion with *HindIII* showed hybridisation with pTi-C58 DNA.

However, the stability of the hybrids formed by different regions varied considerably (data not shown). These results indicate that the two types of Ti-plasmids diverged from each other, conserving some regions more strictly than others.

To determine more precisely the extent of the homology in the fragments pTi-ACH5 *HindIII*-1 and pTi-C58 *EcoRI*-1, with which the dominant hybridisation was observed, the *HindIII* fragment 1 was cloned (G. De Vos *et al.*, in preparation), labelled and hybridised against the *EcoRI* restriction fragments of pTi-C58. As only the *EcoRI*-1 band of pTi-C58 became radioactive after hybridisation (Fig. 2), it must contain all the DNA homologous to the pTi-ACH5 *HindIII* fragment 1. Furthermore, hybridisation against *SmaI* and *HindIII* restriction fragments of the same pTi-C58 DNA indicated that the *HindIII* fragment 1 of the octopine Ti-plasmid was at least partly homologous to the *SmaI* fragments 5 and 7 (Fig. 2 A, c and A, d) as well as to the *HindIII* fragments 22 (2×10^6 daltons) and 31 (1.1×10^6 daltons) of the nopaline Ti-plasmid C58 (Fig. 2, A, e and A, f). *HindIII* fragments with an identical size and belonging to this conserved region were also found in pTi-K14 (Fig. 2 B, e and B, f) and pTi-T37. A more precise estimate of the size of this 'conserved' DNA sequence can be derived from the sum of the two *HindIII* fragments (together 3.1×10^6 daltons) of the nopaline Ti-plasmid, that hybridised with the pTi-ACH5 *HindIII*-1 fragment. A comparison of the physical maps of

octopine and nopaline Ti-plasmids in the region of the 'conserved' DNA segment (see Fig. 4) shows that the homology between the *HindIII* fragments 22 and 31 of the C58 pTi-plasmid and the *HindIII* fragment 1 of the ACH5 plasmid is restricted to the left end of this fragment. The remaining right part (4.9×10^6 daltons) of the pTi-ACH5 *HindIII* fragment 1 does not show any homology with the DNA of nopaline Ti-plasmids.

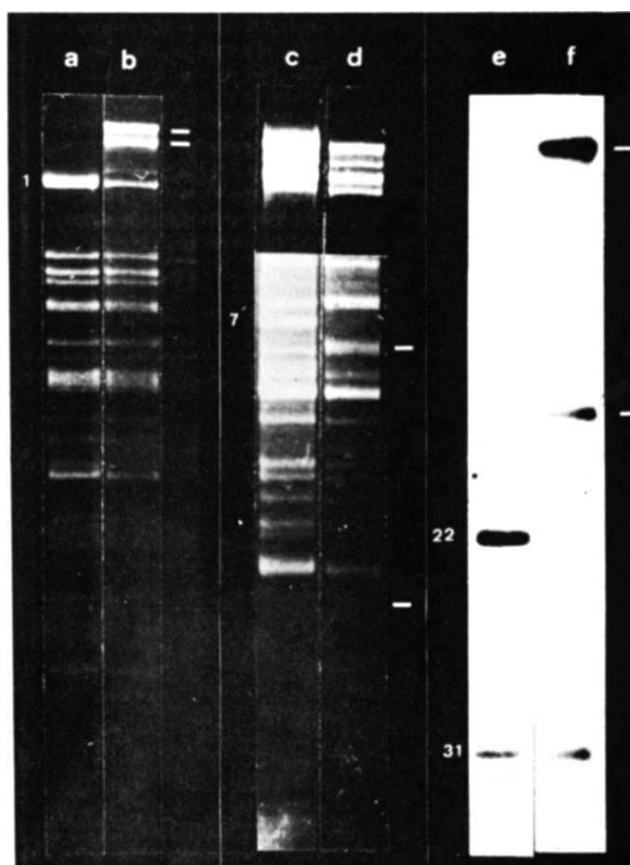
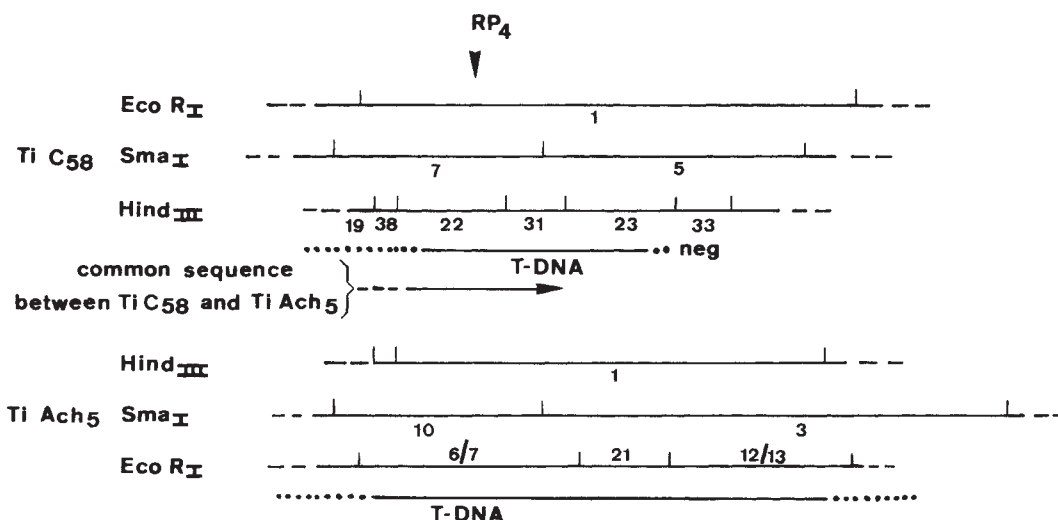


Fig. 3 Localisation of the RP4 integration site in the conserved DNA of the non-oncogenic pTi-C58::pRP4 cointegrate pGV4000. The restriction patterns and hybridisations were carried out as described in the legend to Fig. 1. The pTi-C58 and pGV4000 *EcoRI* (a,b) and *SmaI* (c,d) restriction patterns are shown in parallel to each other. In lanes a and c, the pTi-C58 fragment, in which RP4 is inserted, is indicated by its number. The composite fragment, provided by the junction between pTi-C58 and pRP4 DNA in pGV4000, are indicated by dashes. Lanes a and f, respectively, show the autoradiograms of the pTi-C58 and pGV4000 *HindIII* restriction patterns, which are transferred to nitrocellulose filters and hybridised with the labelled pTi-ACH5 *HindIII* fragment 1 and cloned in the vector pBR322 (ref. 23). The band indicated by an asterisk in lane e is the result of a partial digestion.

Fig. 4 Schematic representation of the conserved region in octopine (pTi-ACH5) and nopaline (pTi-C58) plasmids, showing the restriction map of the corresponding regions in both types of Ti-plasmid. Only those restriction sites used in the present analysis are shown. The cloning, detailed restriction map of the pTi-C58 *EcoRI* fragment 1 and the evidence for the precise localisation of the RP4 integration site in pGV4000 will be described elsewhere (M. De Wilde *et al.*, in preparation). The T-DNA is defined as that segment of the Ti-plasmid that has been found integrated in the DNA of transformed plant cells. Arguments for the extent and localisation of the octopine T-region and for the relevant restriction map were taken from Chilton *et al.*^{16,24} and were adapted according to instructions from M.-D. Chilton. Arguments for the extent and localisation of the nopaline T-region were obtained in our laboratory and will be described elsewhere (De Beuckeleer *et al.*, in preparation).



The results described by Chilton *et al.*²⁴ indicate that the conserved region extends for another 3.5×10^6 daltons to the left of the *HindIII* fragment 1 of the octopine Ti-plasmids.

To discover whether or not this conserved DNA segment was involved in the oncogenic properties of the Ti-plasmids, we used our previous observations¹⁹, that the integration of a complete P-type plasmid pRP4 into the pTi-C58 plasmid can result in a loss of oncogenicity. Dissociation of the non-oncogenic pTi-C58::RP4 cointegrate (pGV4000) into its Ti and RP4 components resulted in a recovery of the oncogenic properties¹⁹. These observations can be explained if one assumes that pRP4 was reversibly integrated into a pTi-DNA segment that is essential for oncogenicity. The *EcoRI* and *SmaI* restriction fragment patterns of pGV4000 indicated that pRP4 had been integrated into the *EcoRI*-1 and the *SmaI*-7 fragments of pTi-C58 (Fig. 3).

To confirm that the integration of pRP4, resulting in a loss of oncogenicity, had occurred in the 'common' DNA sequence, the pTi-ACH5 *HindIII* fragment 1 was hybridised with the *HindIII* restriction fragments of pTi-C58 and pGV4000 DNA. Figure 3 e and f shows that the integration of pRP4 has occurred into the *HindIII* fragment 22 of the pTi-C58, as this fragment is absent and gives rise to two pTi-C58::RP4 composite DNA fragments. As this fragment 22 is completely homologous to the corresponding pTi-ACH5 DNA, one can conclude that RP4 has indeed been integrated into the conserved segment. A more precise mapping of the pRP4 insertion site (data not shown) has confirmed its localisation within the DNA region homologous to all Ti-plasmids tested so far (see also Chilton *et al.*²⁴). Figure 4 shows the position of the *SmaI*, *EcoRI* and *HindIII* fragments of pTi-C58 and pTi-ACH5 in the region of the conserved segment and the localisation of the pRP4 integration site in pGV4000. The T-region is defined as that segment of the Ti-plasmid that has been found integrated into the plant DNA. The extent and localisation of the 'octopine' T-DNA, determined by *Cot* analysis¹⁶ and the 'nopaline' T-DNA, determined by Southern hybridisation (M. De Beuckeleer, in preparation), can be seen in Fig. 4.

The results presented here indicate that different types of Ti-plasmids have a highly conserved DNA segment and that at least part of this conserved or common DNA segment is involved in determining the oncogenic properties of the Ti-plasmids, as the reversible integration of the pRP4 into this

conserved region results in the reversible loss of oncogenicity. The suggestion of the involvement of this conserved segment in oncogenicity is further supported by the finding that it borders and partly overlaps the regions of both the octopine and the nopaline Ti-plasmids (T-DNA) shown to be present in transformed plant cells¹⁶ (De Beuckeleer *et al.*, in preparation). Some of these results have been previously reported in a preliminary form^{10,15}.

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ANN DEPICKER

MARC VAN MONTAGU

JOZEF SCHELL

Laboratorium voor Genetica en voor
Histologie en Genetica,
Rijksuniversiteit Gent,
Ledeganckstraat 35,
B-9000 Gent, Belgium

Received 3 May; accepted 24 July 1978.

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Different cap 1:cap 2 ratios in rabbit α and β globin mRNA

CAP structures type 0 ($m^7G^{5'}ppp^{5'}X$), type 1 ($m^7G^{5'}ppp^{5'}XmY$), and type 2 ($m^7G^{5'}ppp^{5'}XmYmZ$), differing in the number of 2'-*O*-methylated penultimate nucleotides, have been found at the 5' termini of many viral and non-viral eukaryotic messenger RNAs¹. The ability to quantitatively remove the $m^7G^{5'}ppp$ -blocking group from eukaryotic mRNAs and to subsequently label their 5' termini with ^{32}P using [γ - ^{32}P]ATP and T_4 polynucleotide kinase has led to the determination of nucleotide sequences present at the 5' termini for rabbit α and β globin mRNA^{2,3} and alfalfa mosaic virus RNA 4 (ref. 4). The first six nucleotides of both rabbit α and β globin mRNA are identical and these two mRNAs each contain a mixture of cap types 1 ($m^7G^{5'}ppp^{5'}m^6AmC$) and 2 ($m^7G^{5'}ppp^{5'}m^6AmCmA$), differing only by the 2'-*O*-methylation of their penultimate C residues². This 5'-end labelling procedure can also be used to determine the relative amounts of cap types for each individual rabbit

print both on the basis of size and base composition². Further limited digestions of each 5'- ^{32}P -labelled T_1 -fragment with either pancreatic ribonuclease (cleaving only after pyrimidines and yielding ^{32}P - m^6AmCp and ^{32}P - $m^6AmCmAcp$) or T_2 ribonuclease (cleaving after both pyrimidines and purines and yielding ^{32}P - m^6AmCp and ^{32}P - m^6AmCmA) were carried out to determine the relative amounts of type 1 and type 2 cap structures present in each mature mRNA molecule.

Figure 1 shows a representative two-dimensional homochromatographic analysis of a pancreatic RNase digest of the ^{32}P - β -globin mRNA 5'-terminal T_1 -fragment purified as described above. The two distinct products which are clearly resolved in this system, ^{32}P - m^6AmCp and ^{32}P - m^6AmCmA , are separated on the basis of both size and base composition⁵. By similar analysis, a T_2 -RNase digest of the same β - T_1 -fragment also resolved two products, ^{32}P - m^6AmCp and ^{32}P - m^6AmCmA (data not shown). By quantitating the radioactivity present in each product, one can determine the cap 1:cap 2 ratio for an individual globin mRNA. The results from various preparations of rabbit α and β globin mRNA differing only in their relative recovery are summarised in Table 1. The cap 1:cap 2 ratio in three different mRNA preparations was consistently two- to threefold higher for rabbit α mRNA than for β mRNA. This result was independent of the relative quantities of α and β mRNA present in various preparations and the method used for separation and measurement of the T_2 or pancreatic digestion products. Also, these results did not reflect a difference in a potentially lower percentage of 5'-labelling of a type 2 cap structure by T_4 polynucleotide kinase due to the additional 2'-*O*-methylation⁶, as this would have been the case for both α and β mRNAs.

The significance of a two- to threefold enrichment of type 1 cap structures in mature rabbit α relative to β globin mRNA is

Table 1 Ratio of c.p.m. cap 1/c.p.m. cap 2 in [$5'$ - ^{32}P]-labelled rabbit α and β globin mRNA

Preparation	α/β	T_2 digest		Analysis		Mean ratio cap 1/cap 2
		A	C	B	C	
I	0.53	α 515/230 (2.24)	—	—	—	2.24
		β 503/420 (1.20)	—	—	—	1.20
II	1.10	α 650/200 (3.24)	2,200/650 (3.40)	770/275 (2.80)	—	3.15
		β 440/320 (1.37)	—	440/295 (1.35)	700/660 (1.06)	1.27
III	0.35	α —	1,390/530 (2.62)	—	1,210/390 (3.10)	2.86
		β —	2,000/1,425 (1.40)	—	2,950/1,735 (1.70)	1.55

Limit digestion of ^{32}P -labelled α and β globin mRNA 5'-terminal T_1 fragments with either T_2 or pancreatic RNase were analysed as follows. A, Thin layer partition chromatography in isobutyric acid-concentrated NH_4OH-H_2O (66:1:33, v/v) (pH 3.7). B, Thin layer partition chromatography in 0.1 M sodium phosphate (pH 6.8) (100 ml) (NH_4) $_2$ SO $_4$ (60 g), and *n*-propanol (2 ml). C, Two-dimensional homochromatography as described in Fig. 1. All three methods of analysis completely resolved both cap 1 and cap 2 structures from T_2 and pancreatic RNase digestions. Thin layer or homochromatography plates were autoradiographed and material detached from the glass by either scraping or removed as solid platelets using a solution of nitrocellulose⁷ and then counted in toluene scintillant⁵. α/β mRNA ratios were determined from counts present in both ^{32}P -labelled α and β 5'-terminal T_1 fragments purified by two-dimensional fingerprinting² of a T_1 RNase digest of unfractionated 5'- ^{32}P -labelled globin mRNA. There was reproducibly a direct correspondence between the relative amounts of radioactivity present in each 5'- ^{32}P -labelled T_1 fragment and the actual relative amounts of α and β globin mRNA present in each mRNA preparation as determined by polyacrylamide gel electrophoresis in both 7 M urea and 98% formamide². Differences in relative recoveries for α and β mRNA in various polysomal RNA preparations were due to procedural variations during phenol extraction of reticulocyte polysomes².

globin mRNA, without having to first purify each mRNA to homogeneity². In this work the cap 1:cap 2 ratios have been determined. The results indicate a two- to threefold enrichment of type 1 cap structures in α relative to β mRNA.

When a mixture of [$5'$ - ^{32}P]-labelled α and β globin mRNA was completely digested with T_1 ribonuclease, the individual α and β [$5'$ - ^{32}P]-labelled T_1 fragments were purified by two-dimensional fingerprintings². The α - T_1 -fragment (^{32}P - $m^6AmC(m)ACUUCUGp$) and the β - T_1 -fragment (^{32}P - $m^6AmC(m)ACUUGp$) resolve from one another in the finger-

not clear. Results obtained by incubating erythropoietic mouse spleen cells with [methyl- 3H]methionine have suggested that newly synthesised α and β globin mRNA may also exhibit similar cap ratio differences.⁸ In the reticulocyte it has been estimated that each molecule of β globin mRNA initiates protein synthesis at a rate 30% faster than α mRNA⁹⁻¹¹. This difference is probably reflected in a difference in their nucleotide sequences at or near the region for initiation of protein synthesis. In this laboratory, it has been shown that there is a strict requirement for the integrity and presence of the m^7G

residue in the 5'-terminal cap structure for translation of rabbit globin mRNA both *in vitro* and *in vivo*¹². It has also been shown that β -eliminated vesicular stomatitis virus and reovirus mRNA have significantly higher rates of ribosome binding than corresponding unmethylated mRNAs in a reticulocyte lysate¹³. Whether the relative amounts of secondary 2'-O-methylations in normally capped α and β globin mRNA help determine their respective ribosome binding rates in the reticulocyte, however, is open to speculation.

In both mouse L cells¹⁴ and Novikoff hepatoma cells¹⁵, it has been shown that cap structures in cytoplasmic mRNA are derived from type 1 cap structures in heterogeneous nuclear RNA (hnRNA) from which a significant proportion is gradually converted to type 2 cap structures in the cytoplasm. Whether the observed differences in amounts of secondary 2'-O-methylations in rabbit α and β globin mRNA are therefore a reflection of their relative age or relative rates of mRNA turnover during erythroid development is currently under investigation. Recent experiments with mature mouse α and β globin mRNA also indicate similar cap 1:cap 2 ratio differences.

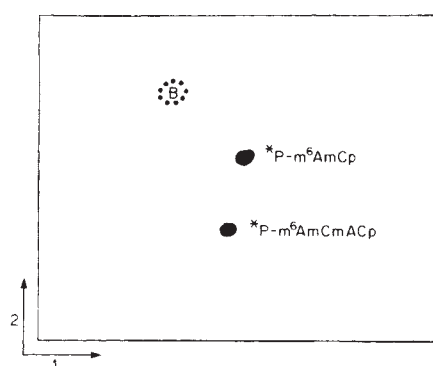


Fig. 1 Autoradiogram of a pancreatic RNase digest of the ^{32}P - β globin mRNA 5'-terminal T_1 -fragment. First dimension: cellulose acetate electrophoresis in pyridinium acetate buffer (pH 3.5) in 7 M urea. Second dimension: DEAE-cellulose TLC in 50 mM KOH strength 'homomix'⁵. The circled 'B' indicates the position of the xylene cyanole FF dye. Preparation of ^{32}P -labelled α - and β -globin mRNA 5'-terminal T_1 fragments was as described elsewhere². Pancreatic and T_2 RNase limit digestion conditions of these fragments and their subsequent analysis by two-dimensional homochromatography has also been described^{2,5}.

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RAYMOND E. LOCKARD

Department of Biology,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

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The murine kappa light chain shift

THE immunoglobulins of mice and other animals consist of several distinct heavy chains associated with either of two light chains (κ or λ). Adult animals of different species produce immunoglobulins which demonstrate widely divergent ratios of κ to λ light chains. In mice, most of B-cell surface immunoglobulins possess κ light chains¹. However, as all previous work on the ratio of κ/λ immunoglobulins was based on experiments with adult animals, the possibility that immature populations of B cells may demonstrate a more evenly balanced ratio of κ to λ positive cells was investigated. In this study, populations of cells known to contain immature B lymphocytes were examined by indirect immunofluorescence for the presence of κ and λ positive cells. The results demonstrate that fetal liver, bone marrow and spleens from young mice contain B cell populations with a κ/λ ratio approaching unity. In the spleen, the κ/λ ratio increased as a function of age with the most dramatic shift occurring between 4 and 6 weeks of age. A similar, but less dramatic increase in the κ/λ ratio occurs in the bone marrow. Based on these data, we propose that the final predominance of κ -bearing cells is based on a two-stage process. Initially, there is an equal probability of κ or λ light chain expression at the cell surface. However, the κ -bearing cells, which possess a more abundant repertoire of V_κ genes, eventually predominate as a result of antigen driven clonal expansion.

Ontogenetic shifts occur between different heavy chain constant region (C_H) genes, allowing the association of the same V region genes with various C_H genes²⁻⁷. In all cases studied, these genes determining the various heavy chains exist in a single linkage group and share a common set of variable region (V_H) genes⁸⁻¹⁴. By contrast, the two classes of light chain, κ and λ , are determined by unlinked genes and each is associated with its own characteristic set of V genes¹⁵. There is, as yet, no evidence of any relationship between light chain class and physiological function or of any ontogenetic shift between λ and κ light chains. Hood *et al.*¹ have suggested that the predominance of κ or λ in a species is due to an evolutionary selection process in which a positive force has preferentially favoured one class of light chain.

It has been observed that the proportion of plasmacytomas and myelomas expressing κ or λ light chains in mice reflects the frequency of those light chains found in normal serum immunoglobulins^{16,17}. Further, it has been observed that the more abundant class of light chain in each species is associated with a greater number of V region subgroups, presumably reflecting a greater number of associated germ-line V genes¹⁶. These observations suggest that the imbalance in the κ/λ ratio is proportional to the number and diversity of the V region genes associated with each.

Here we present evidence that the expression of either κ or λ at the cell surface is a two-step process. The initial choice between expression of κ or λ is based purely on random selection of an equal number of constant region genes. The preferential display of one or the other is then most likely based on antigen driven clonal selection, which in turn is

determined by the number of κ and λ V genes. Furthermore, we believe this selection force occurs during ontogeny, rather than as a phylogenetic event.

The percentage of cells in the spleen, bone marrow, and fetal liver which bear surface κ and λ light chains has been determined by indirect immunofluorescence. As shown in Table 1, the absolute percentage of Ig-positive cells increases in the spleen as a function of age; however, the ratio of κ - to λ -positive cells does not remain constant during this process. Initially, in the spleen of 1-week-old mice, the ratio (κ/λ) is 2.03, yet by 6 weeks of age this ratio has reached 6.59. The shift is not gradual as one might expect, but dramatically changes between 4 and 6 weeks of age. This shift seems to be T-cell independent in that the κ/λ ratio in adult nude mice does not significantly differ from normal adult mice (data not shown). By contrast, the κ/λ ratio in the bone marrow remains near unity until 1 yr of age when the value increases slightly. Similarly, the κ/λ ratio of Ig-positive cells in the fetal and newborn liver approaches unity. A plot of the κ to λ ratio in

the bone marrow and spleen as a function of age is shown in Fig. 1.

Statistical analysis of these data confirms the significance of the apparent increase in the κ/λ ratio (Table 2). In the spleen population, a clear pattern emerges. The κ/λ ratios of animals 1, 2 and 4 weeks old are not statistically different, however, the κ/λ ratios of the 6-week and 1-yr-old mice do differ from the 1–4-week-old groups. Although the κ/λ ratio in the spleen seems to increase again between 6 weeks and 1 yr, the difference was not significant ($P > 0.05$). The differences in the ratios of the spleen populations have been further confirmed by non-parametric Mann-Whitney *C*-sample and one-way analysis of variance testing (see Table 2, legend). The results from these statistical analyses and the apparent trend for the κ/λ ratio in the spleen to increase as a function of age (Fig. 1) support our hypothesis that the κ/λ ratio shifts during ontogeny as a result of some positive selection force, possibly antigenic stimulation. Analysis of the results from the bone marrow population also suggest an increase in the κ/λ ratio as a

Table 1 % κ -positive and λ -positive cells in lymphoid organs as a function of age

Age	Cell population	No. tested	% Stained cells/Total nucleated cells*			Ratio $\kappa:\lambda$
			R $\propto$ MIg	R $\propto$ κ	R $\propto$ λ	
Fetal (18–19d)	Liver	3 pools†	16.7 $\pm$ 1.5‡ (15–18)	10.3 $\pm$ 2.1 (8–12)	9.0 $\pm$ 2.0 (7–11)	1.14
	Thymus	3 pools	2.3 $\pm$ 0.6 (2–3)	ND	ND	
Newborn (<24 hr)	Liver	3 pools	16.3 $\pm$ 2.3 (15–19)	7.7 $\pm$ 2.5 (5–10)	9.3 $\pm$ 3.2 (7–13)	0.83
	Thymus	4 pools	2.8 $\pm$ 0.5 (2–3)	ND	ND	
1 week	Spleen	3 pools	14.0 $\pm$ 2.0 (12–16)	9.7 $\pm$ 0.6 (9–10)	4.7 $\pm$ 1.2 (4–6)	2.06
	Thymus	3 pools	2.0 $\pm$ 1.0 (1–3)	ND	ND	
2 weeks	Spleen	4	20.5 $\pm$ 6.9 (11–27)	14.0 $\pm$ 4.1 (10–18)	4.0 $\pm$ 1.4 (3–6)	3.50
	Thymus	4	1.0 $\pm$ 0.8 (<1–2)	ND	ND	
	Bone marrow	3 pools	24.3 $\pm$ 3.5 (21–28)	8.3 $\pm$ 2.1 (6–10)	13.0 $\pm$ 5.6 (7–18)	0.64
4 weeks	Spleen	5	42.6 $\pm$ 1.5 (41–45)	33.4 $\pm$ 5.8 (26–40)	11.0 $\pm$ 4.1 (6–15)	3.03
	Thymus	4	0.3 $\pm$ 0.5 (<1–1)	ND	ND	
	Bone marrow	4	22.8 $\pm$ 4.4 (19–29)	10.5 $\pm$ 1.9 (9–13)	11.3 $\pm$ 4.8 (7–18)	0.93
6 weeks	Spleen	4	51.8 $\pm$ 5.2 (48–59)	45.5 $\pm$ 5.3 (41–53)	6.9 $\pm$ 1.3 (5–8)	6.59
	Thymus	4	1.3 $\pm$ 1.0 (<1–2)	ND	ND	
	Bone marrow	5	25.6 $\pm$ 2.8 (21–28)	12.8 $\pm$ 3.0 (8–16)	10.2 $\pm$ 5.3 (4–17)	1.25
1 yr	Spleen	8	49.4 $\pm$ 3.0 (46–54)	44.5 $\pm$ 3.9 (41–52)	5.6 $\pm$ 2.3 (3–9)	7.95
	Thymus	4	2.0 $\pm$ 1.4 (1–4)	ND	ND	
	Bone marrow	4	26.8 $\pm$ 5.0 (22–32)	18.5 $\pm$ 6.4 (13–25)	9.3 $\pm$ 3.0 (6–13)	1.99

* Indirect immunofluorescence was performed by a modification²¹ of the method of Pernis *et al.*²². Rabbit anti- κ chain serum (R $\propto$ κ) was from Bionetics and rabbit anti- λ chain serum (R $\propto$ λ) was provided by Dr James D. Folds (UNC, Chapel Hill). Polyspecific rabbit anti-mouse immunoglobulin serum (R $\propto$ MIg) was produced as described previously²³. Fluorescein-conjugated goat anti-rabbit Ig was from Meloy Laboratories. All antisera were used at a dilution of 1:40 in PBS-0.05M NaN₃. Controls for phagocytosis and nonspecific adherence of Ig consisted of cells incubated only with the second antiserum or by using normal rabbit serum in place of the primary antiserum. These were consistently found to be negative (<2%). Results are expressed as % cells positive for surface fluorescence/total nucleated cells counted (>200). Mice of the C57BL/10ScSn and CBA/J background were used. As there was no apparent strain differences all data were pooled for analysis.

† A 'pool' consisted of the spleens, thymuses, or bone marrows of 4 animals which were combined in order to obtain sufficient cells to perform the assay.

‡ Mean $\pm$ standard deviation and range of percentages obtained from determinations for each cell population. ND, not determined.

Table 2 Statistical analysis of change in κ/λ ratio in splenic and bone marrow B lymphocytes during ontogeny: paired contrast *t*-testing

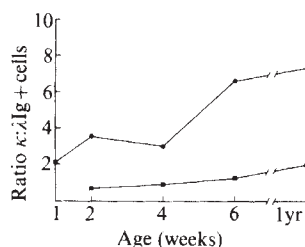
	Age	No. of observations	$\bar{R}$	Statistical difference from:				
				1 week	2 weeks	4 weeks	6 weeks	1 yr
Spleen	1 week	3 pools	2.14 ± 0.43*	X				
	2 weeks	4 pools	3.83 ± 1.64	NS†	X			
	4 weeks	5 pools	3.54 ± 1.91	NS	NS	X		
	6 weeks	4 pools	6.92 ± 1.42	$P < 0.005$	$P < 0.025$	$P < 0.01$	X	
	1 year	8 pools	9.25 ± 4.1	$P < 0.0005$	$P < 0.0005$	$P < 0.0005$	NS	X
Bone marrow	2 weeks	3 pools	0.62 ± 0.31	—	X			
	4 weeks	4 pools	1.01 ± 0.28	—	NS	X		
	6 weeks	5 pools	1.53 ± 0.86	—	NS	NS	X	
	1 year	4 pools	2.07 ± 0.65	—	$P < 0.025$	$P < 0.025$	NS	X

Spleen and bone marrow cells from mice of various ages were assayed for % κ -positive and % λ -positive cells and κ/λ ratio was computed for each observation. For statistical analysis, the mean of the individual κ/λ ratios was determined for each age category. This convention was chosen since comparing the ratios of means is a less well-established procedure for this type of multi-level experiment. For parametric testing it is assumed that these observations are normally distributed as there is no apparent reason to indicate otherwise. Results from the paired contrast *t* test²⁴ are presented above. Results were also tested for significance by one-way analysis of variance (ANOVA)²⁴ and non-parametric Mann-Whitney *C*-sample testing²⁵. On ANOVA testing, the means of the κ/λ ratios in the different age categories of the spleen were found to be statistically different ($P < 0.001$). Similarly when tested by the more conservative non-parametric Mann-Whitney *C*-sample test, these ratios were also found to differ ($P < 0.001$). Although the paired contrast *t* test demonstrates significance for the different age groups in the bone marrow, the means of the bone marrow age groups were not different ($P > 0.05$) when tested by ANOVA and Mann-Whitney *C*-sample testing. Further, it should be noted that these cell populations do not demonstrate homoscedasticity (equal dispersion within age categories), thus the results from these statistics may be affected by failure to comply with this assumption.

* Mean of the individual κ/λ ratios ± s.d.

† Not significant at $\alpha = 0.05$.

function of age. When the means of the ratios for the different age categories were compared by paired contrast *t*-testing, the 2, 4 and 6-week-old groups were not different, however the 1-yr-old group was found to be different from the 2 and 4-week-old groups. Unlike the results obtained with the spleen populations, we were unable to confirm the significance of the changes in the bone marrow with one-way analysis of variance and non-parametric Mann-Whitney *C*-sample testing. Despite these limitations, a general trend towards an increasing κ/λ ratio is seen in the bone marrow as a function of age, suggesting a κ/λ shift occurs which is similar to, but less dramatic than, the shift seen in the spleen.

**Fig. 1** Shift in κ/λ ratio in murine B lymphocytes with age.

Barandun *et al.*¹⁸ have also reported a slight but significant shift in the κ/λ ratio in normal human serum immunoglobulins as a function of age. Also similar to our findings, Spear *et al.*¹⁹ have shown a near doubling in the percentage of Ig-positive Swiss-L mice spleen cells between 2 and 4 weeks of age. Coincident with the increase in B cells, they noted an onset of the capacity to respond to antigens and a significant increase in the ratio of B to T lymphocytes. These events are thought to occur as a result of, rather than the cause for, immunological competence.

Based on these data, we propose that prior to an antigen driven selection process the probability of a cell expressing

surface κ or surface λ is equal. The nearly equal proportion of κ - and λ -positive cells in the populations which contain immature B cell precursors (bone marrow and fetal liver), strongly supports this hypothesis. Between 2 and 4 weeks of age an overall increase in the percentage of Ig-positive spleen cells occurs, yet the relative κ/λ ratio remains essentially the same. However, between 4 and 6 weeks of age, this ratio increases significantly (Table 2). This event may be due to the onset of immunological competence in the animal, allowing the selective clonal expansion of κ -bearing B cells. Molecular studies of immunoglobulin diversity also support this idea. In mice, which have so many more *V* genes associated with and available as partners to the κ *C* gene, the probability that they can collaborate with *V_H* genes to provide a good 'fit' for antigen is high¹⁶. Thus, during maturation, exposure and response to antigens will favour the expansion of the clones of cells expressing κ light chains.

As a corollary to our hypothesis, we would predict that the examination of spleen cells of adult gnotobiotic mice would show a κ/λ ratio significantly different from their normal littermates (that is, closer to unity). Furthermore, it may be possible to shift the κ/λ imbalance prematurely by deliberate immunisation of 2–3-week-old mice. Data from such experiments have proven suggestive, but not significant. The injection of a few exogenous antigens may be inconsequential in relation to the vast array of endogenous and environmental antigens 'seen' by the animal when immunological competence occurs.

The stimulus for this study was the observation that two recently discovered murine B-cell lymphomas were both found to express λ light chain associated with their surface IgM²⁰. This seemed to be most unlikely, on the basis that only about 5% of mouse immunoglobulins express λ chains and only very few murine myelomas have λ light chains^{16,17}. However, other cell surface characteristics (the absence of IgD and C'3 receptors) suggested that both tumours had been derived from a population of cells early in the ontogenetic pathway of B cells²⁰. Thus, we were led to examine other populations of immature B cells. Having done so, it seems that the initial selection made during maturation is between κ and λ *C* genes, and that only later do *V* genes become influential (that is, at

the time of onset of immunological competence). As a result, we further predict that in all species, the proportion of malignancies of 'early' B cells expressing surface κ or λ light chains will approach unity.

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GEOFFREY HAUGHTON

LEWIS L. LANIER

GEORGE F. BABCOCK

University of North Carolina School of Medicine,
Department of Bacteriology and Immunology,
Chapel Hill, North Carolina 27514

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Comparison of the evolution rates of cytosolic and mitochondrial aspartate aminotransferase

TWO isoenzymes of aspartate aminotransferase (AAT) occur in animal and possibly in all eukaryotic cells, one in the cytosol (cAAT) and the other in the mitochondria (mAAT)¹. Both isoenzymes are composed of two identical polypeptide chains of about 400 amino acid residues and their reaction mechanisms and kinetic features are essentially the same^{1,2}. They are homologous proteins, as evidenced by 48% sequence identity of the two isoenzymes from pig^{3,4}, and are both coded for by nuclear DNA and synthesised by cytosolic ribosomes⁵⁻⁷. Comparison of the isoenzymes from pig with those from chicken by quantitative microcomplement fixation has shown that the interspecies similarity of the mitochondrial isoenzymes markedly exceeds that of the cytosolic isoenzymes⁸. The same conclusion has been reached by comparison of the known amino acid sequences of the pig isoenzymes^{3,4} with the chicken isoenzymes, for which more than half of their sequences have already been determined. The sequence identity of the two cytosolic isoenzymes seems to be approximately 50% (Y. M. Torchinsky, personal communication), whereas that of the two

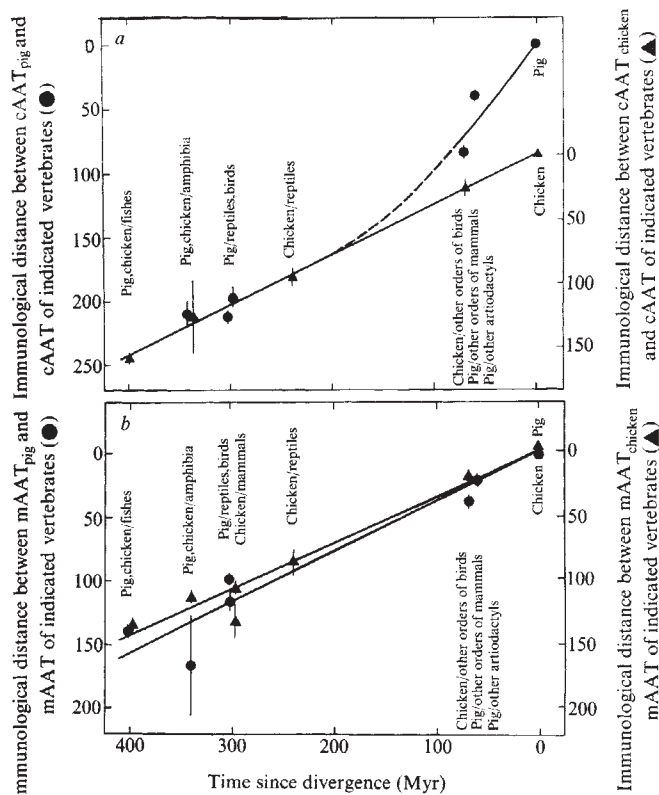


Fig. 1 Evolution of cytosolic (a) and mitochondrial (b) AAT. The mean immunological distances between the reference enzymes from pig and from chicken and the respective isoenzymes from the indicated vertebrate classes or orders are plotted against the palaeontologically estimated times of divergence of the ancestors of the reference species pig and chicken from the various vertebrate classes or orders. Immunological distances are taken from Table 1 and are not corrected for multiple mutations at the same site. To join the lines of the cytosolic pig and chicken enzyme in a at the point of their divergence 300 Myr ago, the line of the porcine enzyme was translated vertically by +83 immunological distance units. This translation distance was determined as the difference between the immunological distance of mammals and birds (mean of the reciprocal crossreactions 200 units) and the interpolated value of the line of the chicken enzyme at 300 Myr (117 units). The assumed times of divergence are¹³, in Myr: fishes, 400; amphibians, 340; birds and reptiles from mammals, 300; birds from reptiles, 240; interordinal divergence of placental mammals, 70; interordinal divergence of birds, 70; pig from the other artiodactyls, 60. The slope of the lines corresponds to the double value of the rate of evolution because the immunological distance measured is the sum of the changes accumulated by the 2 species compared since divergence from a common ancestor. The slope of the line of cAAT is 0.39 ($r=0.95$) throughout the vertebrate classes except for mammals, where the slope is 0.97. For mAAT, the slope is 0.39 ($r=0.96$) and 0.36 ($r=0.98$) when measured with anti-mAAT_{pig} antiserum and with anti-mAAT_{chicken} antiserum, respectively.

mitochondrial isoenzymes is about 85% (ref. 9 and U. Hausner, P. C., K. J. Wilson, unpublished). In the present study, the immunological interspecies comparison of the two isoenzymes was extended to species of all vertebrate classes. The evolution of the two compartmented isoenzymes was found to have proceeded at identical and constant rates throughout the development of the nonmammalian vertebrates. After the emergence of mammals, however, the rate of evolution of the cytosolic isoenzyme more than doubled while its mitochondrial counterpart retained the slower rate of the nonmammalian vertebrates.

Table 1 Immunological distance between cAAT and mAAT from pig and chicken and the respective isoenzymes from species of all vertebrate classes

Species (source of extract)	Immunological distance between reference enzyme and respective isoenzyme from the indicated species			
	cAAT _{pig}	mAAT _{pig}	cAAT _{chicken}	mAAT _{chicken}
Artiodactyls				
Pig (<i>Sus scrofa</i>)	0	0	218	108
Black-Buck (<i>Antilope cervicapra</i>)	45	30	177	120
Sheep (<i>Ovis ammon</i>)	33	22	162	98
Ox (<i>Bos taurus</i>)	30	13	178	102
Stag (<i>Cervus elaphus</i>)	41	24	186	100
Alpaca (<i>Lama guanacoë</i>)	49	28	187	110
Mean $\pm$ s.e.m.	40 $\pm$ 4	23 $\pm$ 3.5 $\pm$ 8	106 $\pm$ 3	
Species of other mammalian orders				
Dog (<i>Canis familiaris</i>)	63	39	189	116
Cat (<i>Felis silvestris</i>)	73	26	224	116
Horse (<i>Equus caballus</i>)	67	40	232	173
Otter (<i>Lutra lutra</i>)	97	52	237	123
Mean $\pm$ s.e.m.	75 $\pm$ 8	39 $\pm$ 5	221 $\pm$ 11	132 $\pm$ 14
Birds				
Chicken (<i>Gallus gallus</i>)	189	102	0	0
Duck (<i>Anas platyrhynchos</i>)	162	98	12	20
Goose (<i>Anser anser</i>)	180	109	18	18
Rhea (<i>Rhea americana</i>)	197	89	7	28
Pigeon (<i>Columba livia</i>)	236	98	10	12
Budgerigar (<i>Melopsittacus undulatus</i>)	207	105	45	43
Buzzard (<i>Buteo buteo</i>)	215	82	48	13
Crow (<i>Corvus corone</i>)	192	119	42	7
Mean $\pm$ s.e.m.	197 $\pm$ 8	100 $\pm$ 4	26 $\pm$ 7	20 $\pm$ 5
Reptiles				
Tortoise (<i>Testudo hermanni</i>)	224	126	104	107
Turtle (<i>Chelonia spp.</i>)	207	110	100	102
Crocodile (<i>Crocodilus niloticus</i>)	215	100	75	73
Python (<i>Python reticulatus</i>)	203	130	100	63
Mean $\pm$ s.e.m.	212 $\pm$ 5	117 $\pm$ 7	95 $\pm$ 7	86 $\pm$ 11
Amphibia				
Frog (<i>Rana ridibunda</i>)	219	127	98	110
Salamander (<i>Pleurodeles walthii</i>)	198	207	154	113
Mean $\pm$ s.e.m.	209 $\pm$ 11	167 $\pm$ 40	126 $\pm$ 29	112 $\pm$ 2
Fishes				
Whitefish (<i>Coregonus fera</i>)	—	135	151	138
Trout (<i>Salmo fario</i>)	—	151	172	132
Carp (<i>Cyprinus carpio</i>)	—	130	158	143
Grayling (<i>Thymallus thymallus</i>)	—	143	160	128
Mean $\pm$ s.e.m.	—	140 $\pm$ 5	160 $\pm$ 4	135 $\pm$ 3

The immunological distance between the reference antigens (isoenzymes from pig and chicken purified to homogeneity) and the corresponding isoenzymes of the tested species were measured by quantitative microcomplement fixation as reported previously⁸. In pig and chicken the immunological distances obtained with heart tissue extracts were the same as those determined previously with the isolated enzymes⁸. No crossreactions between isolated cAAT and mAAT from either pig or chicken were observed. Thus, heart tissue extracts were tested without separating the two isoenzymes. Heart tissue was frozen not later than 3 h post mortem and stored at -20°C . The thawed tissue was homogenised with a Duall homogeniser in 5 volumes of the buffer used for the complement fixation, and centrifuged at 2,000g for 15 min. The extent of complement fixation at different concentrations of antiserum was determined at serial dilutions of the homologous and the heterologous antigen. A plot of the peak heights of the complement fixation curves against the logarithm of the antiserum concentration yielded a straight line. The lines of the crossreactions of a given antiserum with heterologous antigens were essentially parallel to the line obtained for the homologous reaction. The immunological distance (*ID*) was calculated according to $ID = 100 \log$ (antiserum concentration for 50% complement fixation with the heterologous antigen/antiserum concentration for 50% complement fixation with the homologous antigen) (refs 8, 10). Both anti-mAAT antisera crossreacted with rabbit heart extract as judged by quantitative microcomplement fixation and the Ouchterlony double-diffusion test. The crossreacting antigen from rabbit heart showed the same electrophoretic mobility in immunoelectrophoresis and the same molecular weight as mAAT from pig and chicken (determined by polyacrylamide gel electrophoresis in sodium dodecyl sulphate after extraction of the antigen-antibody complexes with *Staphylococcus aureus* carrying protein A). Thus, it is very likely that the immunisation with either heterologous mitochondrial isoenzyme had resulted in the production of antibodies against the rabbit's own mAAT. Similar breakage of the immunological tolerance has been reported for α -fetoprotein¹¹. In the present instance, this phenomenon might be related to the sequestration of the antigen in the mitochondria. The crossreactions in quantitative microcomplement fixation between the rabbit mAAT and the rabbit antisera against mAAT from pig and chicken are weak, corresponding to an immunological distance of 211 and 206, respectively. These crossreactions reflect an increase in antigenicity of the mitochondrial isoenzymes, that is, a larger surface portion surveyed by the antibodies, which may be expected to increase the reliability of the immunology sequence relationship¹². No reliable crossreactions were obtained from anti-cAAT_{pig} antiserum and the fish heart extracts.

Antisera to cAAT and mAAT from both pig and chicken were produced as described previously⁸. The degree of immunological crossreactivity of these reference antisera with the respective isoenzymes from representatives of all vertebrate classes was determined by quantitative microcomplement fixation and was expressed in terms of immunological distance (Table 1). Remarkably, the immunological distances between cAAT_{pig} and cAAT from all other species are substantially larger than those between mAAT_{pig} and mAAT from the other species. Similarly, the immunological distances between cAAT_{chicken} and cAAT from mammalian species are twice as large as the corresponding immunological distances obtained for mAAT_{chicken}. In contrast, the same immunological distances were obtained when cAAT_{chicken} and mAAT_{chicken} were compared with the corresponding isoenzymes from the non-mammalian vertebrate classes. The temporal course of the evolution of the cytosolic and the mitochondrial isoenzymes is reconstructed in Fig. 1a and b, respectively. The plots of the immunological distances against the times of divergence of the phylogenetic lineages show a constant rate of change for mAAT throughout all vertebrate classes. A constant rate of the same magnitude applies also to cAAT in all nonmammalian vertebrates. However, during the development of mammals, the evolution of the cytosolic isoenzyme proceeded more than twice as fast, indicating that an increase in the rate of change had occurred after the mammals had diverged from the common ancestor of reptiles and birds. This acceleration of the rate of evolution as found with the anti-cAAT_{pig} antiserum is confirmed by the reciprocal reactions with anti-cAAT_{chicken} antiserum (see Table 1).

For a set of homologous variants of a given protein, the immunological distance (ID) has been found to correlate linearly with the percentage amino acid sequence difference (Δaa) according to the equation $ID \approx 5 \times \Delta aa$ (refs 14, 15). For cAAT, the average time required for a 1% change in the amino acid sequence to arise between two genealogical lineages can thus be estimated to be 6 Myr in mammals and 12 Myr in the other vertebrates. The latter estimate also obtains for mAAT throughout the phylum of vertebrates. The values lie within the range found for other intracellular enzymes¹⁶. On the basis of these rates of change, the gene duplication underlying the development of the mitochondrial and the cytosolic isoenzyme (amino acid sequence difference in pig, 52% (ref. 4)) may be assumed to have taken place about 600 Myr ago, at the time of the emergence of the vertebrates. This indicates that the isoenzymes of the vertebrates do not originate from the same gene duplication that gave rise to the isoenzymes found in fungi¹⁷ and plants¹⁸.

Changes in the rate of evolution of certain proteins such as globin chains¹⁹ or lysozyme/lactalbumin²⁰ have been reported, but such changes have been questioned¹⁶. However, the main argument against these cases, that of the uncertainty in estimating the time of interspecies divergence, does not seem pertinent to aspartate aminotransferase. The increase in rate is found specifically with the cytosolic isoenzyme, whereas the closely related mitochondrial isoenzyme retains the slower rate of evolution. Thus, the conclusion that the cytosolic isoenzyme changed its rate of evolution is warranted independently of the palaeontological time scale.

The increase in the rate of evolution of the cytosolic isoenzyme in mammals may be due to positive natural selection towards an improved, newly developed function of the isoenzyme or may reflect the loss of an obsolete noncatalytic function. The cause of the change in the evolution rate remains open to conjecture. The fact, however, that the crucial difference between the two compartmented isoenzymes exists only in mammals might facilitate its eventual identification. Data soon available on the primary and spatial structure of both isoenzymes²¹⁻²³ will enable the comparative study of the evolution of their structural and functional features, thus providing an informative approach to the study of the interrelationship of function and evolution of proteins.

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PETER SONDEREGGER

PHILIPP CHRISTEN

Biochemisches Institut
der Universität Zürich,
Zürichbergstrasse 4,
CH-8028 Zürich, Switzerland

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Structure of DNA predicted from stereochemistry of nucleoside derivatives

CRYSTALLOGRAPHIC data on the constituents of nucleic acids can be very useful in predicting secondary structures. Until recently only data from the crystal structures of mononucleosides and mononucleotides have been used in building models of secondary structures for nucleic acids and polynucleotides. However, such data only provide information about the stereochemistry of nucleotides and not the relative orientation of the nucleotides around the 3' and 5' phosphodiester bonds. This relative orientation is the key factor which determines the secondary structure of nucleic acids and details of this orientation can be obtained from single-crystal study of dinucleoside monophosphates and other higher oligomers. So far, crystal structure data on the dinucleoside monophosphates GpC (sodium salt)¹, GpC (calcium salt)², ApU³, UpA⁴, a dinucleotide pTpT⁵, a trinucleoside diphosphate ApApA⁶, and a tetranucleotide pApTpApT (M.A. Viswamitra, personal communication) are available. We have analysed the conformations of these molecules and present here stereochemical criteria for the backbone of the polynucleotide chains and the nucleic acids. Application of these criteria to nucleic acids indicates type II structure for DNA, as proposed previously by us⁷ as an alternative to the double helix.

Table 1 lists in the alphabetical notation the torsion angles of the sugar phosphate backbone of dinucleoside monophosphates.

Table 1 Backbone torsion angles for the fragments from dinucleoside monophosphates and related molecules

		$\zeta(5')$ C4'-C3'	α C3'-O3'	β O3'-P	γ P-O5'	δ O5'-C5'	ϵ C5'-C4'	$\zeta(3')$ C4'-C3'	Ref.
Set I	GpC	89	211	292	285	184	50	77	1
	GpC1	79	222	294	291	181	47	79	2
	GpC2	73	217	291	293	172	57	80	2
	GpC3	96	224	290	286	167	63	74	2
	GpC4	88	216	288	283	181	52	87	2
	ApU1	84	213	293	288	177	57	74	3
	ApU2	78	221	284	295	168	58	77	3
	ApA ⁺	82	223	283	297	160	53	81	6
	ApT [†] (1)	90	213	294	293	176	68	134	†
Set II	UpA(2)	77	224	164	271	192	54	93	4
	TpT [†]	157	252	163	288	187	41	158	5
	TpA [†]	134	204	168	286	186	49	83	†
Set III	UpA(1)	86	206	81	82	203	55	85	4
	A ⁺ pA ⁺	81	207	76	92	186	56	79	6
Set IV	A RNA	95	202	294	294	186	49	95	14
	A DNA	83	178	313	285	208	45	83	15
	B DNA	156	155	264	314	214	36	156	16
	C DNA	141	211	212	315	143	48	141	17
	D DNA	156	141	260	298	208	69	156	18
	B DNA (left)	140	200	212	282	165	40	140	‡

* Deoxy sugar.

† M. A. Viswamitra, personal communication.

‡ Present work.

phates and similar fragments in higher oligomers³: row 2 shows the respective bonds about which the torsion angles are measured. The sugar and nucleoside parts of these dinucleoside monophosphate fragments have conformational features similar to those of mononucleosides and nucleotides⁸. Thus

puckering of the sugar is either in the C3' *endo* or C2' *endo* region. The conformation about C5'-O5' is *trans* (*t*) and about C4'-C5' is *gauche-gauche* (*gg*). The major degrees of conformational freedom responsible for the orientation of the adjacent nucleotide units are about the phosphodiester bonds and accordingly these fragments have been grouped into three sets. Sets I, II and III have, respectively, the conformation g^-g^- , tg^- , g^+g^+ about the P-O bonds.

In sets I and II, γ varies from 271° to 302°, whereas β varies from 283° to 293° in set I and 163° to 168° in set II. In set III both β and γ are restricted from 76° to 92°. Thus for β , the observed conformations correspond to g^- , t , g^+ , whereas for γ , they are g^- and g^+ . The other torsion angle α varies from 204° to 224° except in TpT where it is 252°. Conformational energy calculations^{9,10} also indicate that tg^- , g^+g^+ conformations about the P-O bonds observed in the crystal structures are energetically as favourable as the g^-g^- conformation. The above conformational criteria of the backbone of the dinucleotide fragments have been used to predict possible secondary structures for polynucleotide chains.

Taking into account the conformational flexibility about the phosphodiester bonds and the possible variations in the remaining torsion angles, the conformational features of regular helical polynucleotide were analysed, using average bond lengths and bond angles for the monomer unit. Both right- and left-handed double helical structures with Watson-Crick base-pairing schemes were studied. The details of the calculations and the results will be reported elsewhere. Here, we discuss first the single chain helical parameters n and h (n is the number of nucleotide residues per turn and h the height per residue) which are relevant to the conformation of double stranded polynucleotides. As stated above, the major degrees of conformational freedom and the key to the conformation of nucleic acids are about the P-O3'(β) and P-O5'(γ) bonds. The salient features of the results are therefore presented in the β - γ space keeping other torsion angles fixed (Fig. 1). Although the crystal data given in Table 1 suggest only *gg* conformation about the C4'-C5' bond, we have also considered *gt* and *tg* conformations about this bond to determine the sensitivity of

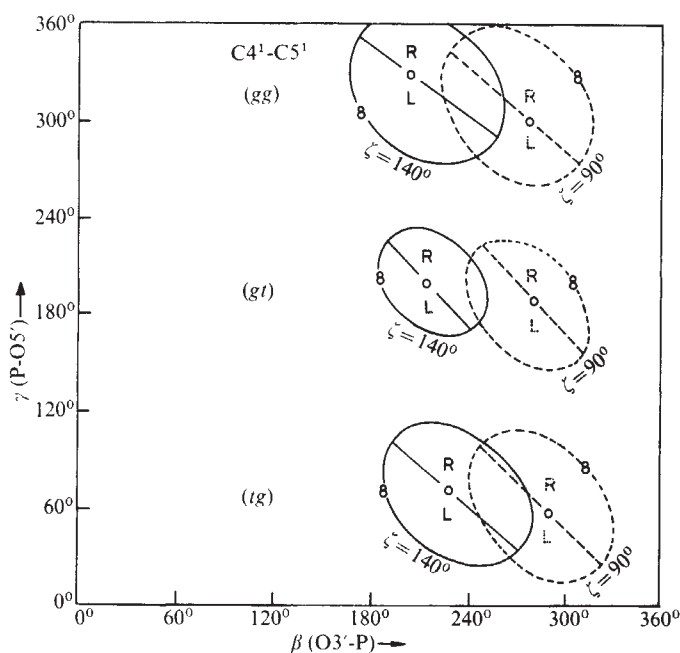


Fig. 1 Helical domains in the β - γ space corresponding to the conformations *gg*, *gt* and *tg* about the C4'-C5' bond, for C2' *endo* (solid line) and C3' *endo* (dashed line) sugar pucker. Only $n = 8$ and $h = 0$ are shown. Right and left helical sections are denoted by R and L.

the helical parameters with these conformations. The results are shown for two representative values of ζ , 90° and 140° ; the former in the C3' *endo* region and the latter in the C2' *endo* region. Interestingly the helix forming domains shown in Fig. 1 contain both right- and left-handed helices, and small changes in the values of β and γ alter the sense of the helix. Also the helix that forms domains in the β - γ space is sensitive to the sugar puckering and the conformation about the C4'-C5' bond. Thus a change in ζ from 90° to 140° produces a shift in β from g^- to t . Thus a change in the conformation about the C4'-C5' bond also produces a corresponding change in the conformation about γ . If the conformations about the C4'-C5' bond are *gg*, *gt* and *tg*, then in the helical domains the corresponding conformations about the P-O5' bond are g^- , t and g^+ respectively.

The observed conformation about the C4'-C5' bond is *gg*, so the helical domains in the β - γ space will be restricted to g^-g^- and tg^- . These are precisely the conformations about the P-O bonds that are observed in the crystals and given under sets I and II of Table 1. When g^+g^+ conformations of set III are used, no regular helical polynucleotide structures can be generated^{11,12}. But these conformations in the middle of a polynucleotide can reverse the chain direction¹³. In other words, g^+g^+ conformations about the P-O bonds can lead to a fold or bend along its backbone. Besides, in the case of a helical polynucleotide, the g^+g^+ conformation in the middle can change the handedness of the helix. We have used this conformation in the bend region of the type II model for DNA⁷.

Set IV in Table 1 lists the backbone torsion angles of right-handed double helical structures for nucleic acids reported in the literature¹⁴⁻¹⁸ for comparison with the crystal data. The agreement between A-RNA¹⁴ and the crystal data is very good. In contrast the agreement between the various conformations of DNA and the observed data is not good. In fact, the agreement is poor for both B and D DNA^{16,18}— α is much lower than the lowest observed value. Also, angles β and ϵ in B DNA and β in D DNA are different from the observed values.

To establish the conformational angles of the backbone for different forms of DNA which are close to the observed angles, helical domains were computed in the α - β space keeping γ , δ

and ϵ fixed in their respective average values (Table 1). The helical domains (also n and h contours) thus obtained for the average values of ζ in the C2' *endo* and C3' *endo* regions are given in Figs 2 and 3. The α , β values given under sets I and II are also plotted in these figures to show roughly the type of helical structures that will be generated for these fragments.

As before, the $h = 0$ contour divides the helical domains into right and left helical sections, and an iso- h contour cuts an iso- n contour at two places. Thus, as shown in the diagram, four regions, R_I and R_{II} in the right and L_I and L_{II} in the left helical sections are possible for single chains. For a given n and h value, in each helical domain two right and two left helical

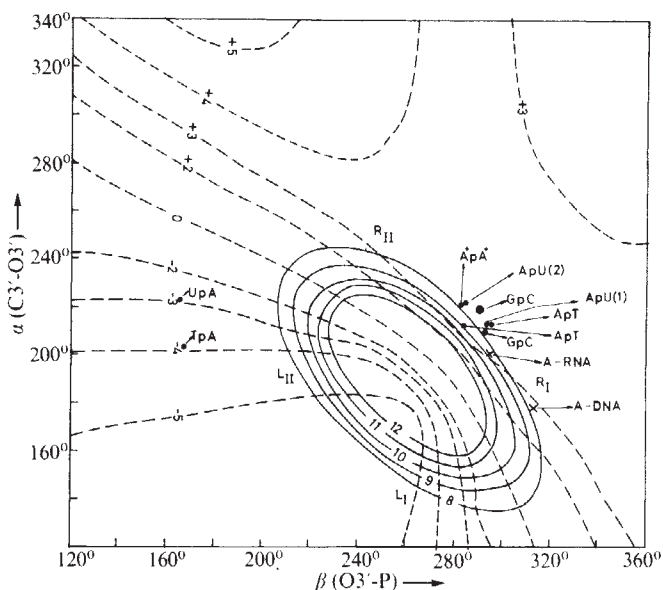


Fig. 3 Helical domains in the α - β space for C3' *endo* puckering ($\zeta = 90^\circ$). n (solid line) and h (dashed line) contours and observed torsion angles α and β of the fragments with C3' *endo* puckering for the sugar are shown. For GpC (Ca) the plotted conformation refers to the average values of the four independent molecules in the crystal.

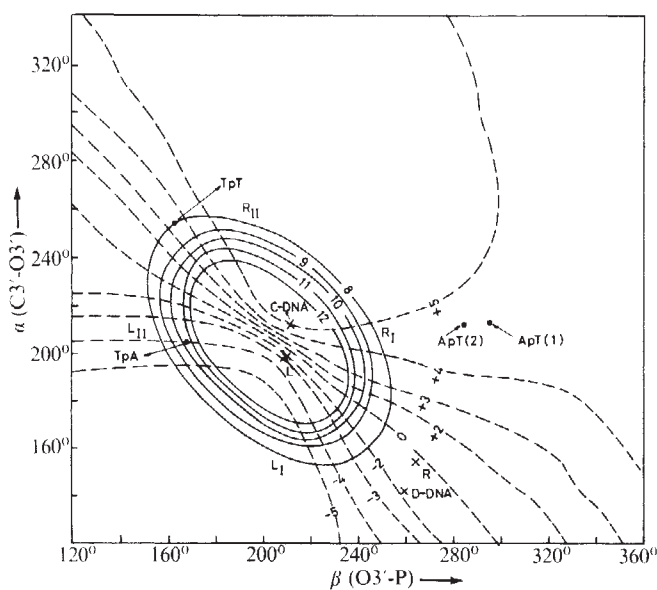


Fig. 2 Helical domains in the α - β space for C2' *endo* puckering ($\zeta = 140^\circ$). n (solid line) and h (dashed line) contours and observed torsion angles α and β of the fragments with C2' *endo* puckering for the sugar are shown. R and L denote right- and left-handed B DNA.

structures are possible. However, not all of them lead to double helical structures with Watson-Crick base pairing.

In the C2' *endo* case shown in Fig. 2, the observed conformations ApT(1) and ApT(2) have very small n values and hence double helices are not possible. TpA and TpT lie in the L_{II} regions. In both these regions the bases are away from the helix axis and hence base-paired double helical structures are again not possible. Thus, double-helical structures are not generated when one uses the observed backbone conformational angles in the C2' *endo* region.

In the R_I and L_I regions, however, the bases overlap and hence double helical structures are possible. In fact by allowing the variations that are observed in the remaining torsion angles one can construct a series of stereochemically feasible right- and left-handed double helical structures consistent with the helical parameters of B DNA. The values of α and β for a left-handed double-helical structure with the B DNA parameters which we have constructed are plotted in Fig. 2 together with those for the right-handed helical structure¹⁶. We are now investigating whether X-ray fibre diffraction data could be used to distinguish between left-handed and right-handed models which have conformational angles as close as

possible to those observed in the crystals. The only remaining conformational angle χ about the glycosidic bond has a value near 0° for the left-handed structure while $\chi \approx 75^\circ$ for the right-handed structure, as reported previously⁷.

In Fig. 3, for the C3' *endo* case, except UpA and TpA, the other observed conformations are clustered in R_1 with $n = 8$ to 11 and $h = 2$ to 3 Å. Thus double helical structures can indeed be generated with the observed backbone conformational angles in the C3' *endo* region. In the case of both L_I and L_{II} regions, the bases overlap, so double helical structures are possible.

Thus for the *gg* conformation about the C4'-C5' bond, the helical domains occur in the β - γ space at the g^-g^- and tg^- regions, which are precisely the observed regions of conformations for the fragments given. Small variations in dihedral angles bring about changes in the sense of the helix and both right- and left-handed double helical structures are possible. It is clear that conformations similar to the fragments in sets I, II and III can lead to right helical, left helical and bend (or fold) structures respectively. A structural model for B DNA can thus be constructed with right and left helical segments having backbone conformations similar to sets I and II respectively. Conformations similar to set III serve to link consecutive right and left segments of a chain. A structure thus arrived at is a type II structure proposed previously by us⁷ as an alternative structure for DNA. The type II structure is not a double helix but consists of a pair of polynucleotide strands held together by Watson-Crick base pairing. Each of the two strands has alternating right- and left-handed segments approximately five base pairs long. As a consequence of mixing right-handed with left-handed helical segments and vice versa, each strand has bends or folds. These bends impart a significant amount of flexibility to the polynucleotide. This inherent flexibility of the type II structure can permit the initiation of the supercoiled structure of DNA following energetically allowed folds. This flexibility along the backbone could provide a possible solution to the compression of DNA within chromatin¹⁹.

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V. SASISEKHARAN

N. PATTABIRAMAN

Molecular Biophysics Unit, Indian Institute of Science,
Bangalore 560 012, India

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Corrigendum

In the article 'Decision making in animals' by D. J. McFarland, *Nature* **269**, 15 (1977), the sentence starting in line 16 on page 18 should read: On this basis we can express the expected probability of fertilisation, given that the male is still involved in the courtship as

$$\varepsilon \int_0^T [u_1(t) + e^{\varphi S(t)t} u_3(t)] S(t)x(t) dt.$$

Also on page 18, the scheme based on Dempster's formulation (ref. 14) should read as shown below:

Objective: Maximise likelihood of successful fertilisation

$$\int_0^\infty \{ \exp [-\varphi S(t)t] S(t)u_1(t)x(t) + S(t)u_3(t)x(t) \} dt$$

Plant equation: $\dot{x}(t) = -x(t)^{-\rho}u_2(t) + \theta$

Work during courtship: $\frac{u_1(t)}{\delta} + \frac{u_2(t)}{\gamma} + \frac{u_3(t)}{\tau} \leq 1$

Energy expended during courtship:

$$\int_0^\infty \left[\frac{u_1(t)}{\delta} + \frac{u_2(t)}{\gamma} + \frac{u_3(t)}{\tau} \right] dt \leq E$$

Hamiltonian:

$$H = e^{-\varphi S(t)t} S(t)u_1(t)x(t) + S(t)u_3(t)x(t) - F(t) \left[-x(t)^{-\rho}u_2(t) + \theta \right]$$

(Costate) Hamiltonian equation:

$$\dot{F}(t) = \frac{\partial H}{\partial x} = e^{-\varphi S(t)t} S(t)u_2(t) - \rho \frac{F(t)}{x(t)^{1+\rho}} u_2(t) + S(t)u_3(t)$$

states: S = spermatophore count (0-3)

x = oxygen 'debt'

controls: u_1 = rate of display behaviour (maximum rate δ)

u_2 = rate of creep behaviour (maximum rate γ)

u_3 = rate of spermatophore transfer (maximum rate τ)

parameters: φ = basic discount rate of display behaviour
 ρ = control parameter for rate of decrease of x through skin respiration during creep behaviour

θ = rate of increase of oxygen debt with time (during display and transfer behaviour)

E = maximal energy expended in courtship

F = costate variable = marginal value of likelihood of success per unit of x

Nature Index and Binders

The complete **Index** for 1977 is available, price £2.50 (UK), US\$5.00 (Rest of World). Copies of the 1976 index are still on sale, prices as above.

Binders for the journal are also available at £8.00 (UK), US\$16.00 (Rest of World) for three: a year of *Nature* fits into three binders.

All prices include postage. Payment may be made in any currency at the prevailing exchange rate. Orders should be sent, accompanied by remittance, to Macmillan Journals Ltd, Brunel Road, Basingstoke, Hampshire, RG21 2XS, UK.

reviews

Coming to terms with Nature

Eric Ashby

The Twenty-Ninth Day: Accommodating Human Needs and Numbers to the Earth's Resources. By L. R. Brown. Pp. 363. (Norton: New York, 1978.) \$11.95.

THE title of this book is another example of the hypnotic effect of exponential and sigmoid curves upon those who have never used them in serious scholarly work. The lily pond which doubles its leaf-area every day and is half-full on the twenty-ninth day will, so the introduction to this book says, be full on the thirtieth day. Had the author taken the trouble to read the brilliant analysis of population growth published by Alfred Lotka over half a century ago in *Elements of Physical Biology*, he might have hesitated before starting his argument with such a misleading example. The dilemma facing mankind is not what to do about exponential growth; it is how to devise political systems which can be used to manage the second-order consequences which follow the point of inflection of a sigmoid curve of growth.

Forecasts of population growth, oil reserves, capacity of the land to produce food, resources of minerals: all these vary by whole orders of magnitude. Disputes about the forecasts miss the point: that all these parameters already are, or sooner or later will be, above the point of inflection of a sigmoid curve; and the general characteristic of this part of the curve is that feedback, which was positive while the curve was convex to the time-axis, now becomes negative.

Discontinuities invariably occur: nations like China successfully limit the birth rate; nuclear power may replace oil for some purposes; plant breeders may increase yield per hectare, aluminium may replace copper. Some of the discontinuities (for example, the substitution of steam-power for water- and wind-power) have profound social consequences, which have been well documented. But, discontinuities apart, the trend is indisputable; exponentials become sigmoid and the seminal social problem is not how to delay the point of inflection by technological innovation, nor is it how to bring forward the point of inflection by rationing; it is how to manage society when many

measurable variables lie on the upper reaches of sigmoid curves.

This is the worthy theme in Lester Brown's book. In the first nine chapters he covers familiar, not to say overcrowded, ground: the evidence for over-fishing, over-grazing, deforestation; the inevitable rise in population; the geopolitics of energy production; the economic stresses which followed a sixfold increase in oil prices imposed by OPEC and (though western countries are more reticent about this) a two-and-a-half-fold increase in wheat prices imposed by the grain-producing nations and a threefold increase in the price of newsprint; and the growing gap between affluence and poverty.

His sources of information for the material in these chapters are practically all from journalistic and sometimes emotive accounts in the press or in popular books. His exposition of these issues is no worse, and no better, than that in a dozen other books covering similar ground. So it is to the last three chapters, which are about how society is to come to terms with Nature, that I turned with some anticipation. For Lester Brown, unlike many middle-class élitists keen on conservation, has really encountered the world-dilemma at close quarters, as an agricultural officer, as a dweller in Indian villages, and with the Overseas Development Council. This experience (I hoped) would dissuade him from advocating the utopian remedies which prompt decision-makers to drop such books into the waste paper basket.

That Lester Brown's three chapters on accommodation to the new phase of world economy do not provide encouraging solutions is not his fault. No one has come up with even the rudiments of a solution. But Lester Brown does put the emphasis where it belongs, namely on the need to find solutions for social and ethical problems rather than material problems. The great disillusion is not directed against the technologists; it is directed against the economists; and he quotes a telling remark made by Pierre Trudeau: "Inflation has not found its Keynes. I personally think the Keynes of inflation will not be an economist . . ." but will instead "be a political, philosophical or moral leader inspiring people to do without the excess consumption so

prominent in the developed countries". The difficulty is that this recipe may be valid for the inhabitants of Dorking and Wimbledon (well heeled suburbs of London) but not for the swarms of people who squat on the streets of Calcutta. Up to a point there is a close correlation between growth in GNP and the overall improvement in quality of life; it is in affluent societies that the correlation breaks down. So—and this is the conclusion that hurts—the initiative lies in Dorking and Wimbledon and not in Calcutta. And the choice before Dorking and Wimbledon is voluntary adjustment to austerities or submission to bureaucratic regulation.

Some writers—Heilbroner, for example, and Ophuls—are pessimistic about the outcome of this choice. They see (as Heilbroner puts it) the latitude for voluntary choice diminishing "under the slowly closing vise of environmental constraint". Lester Brown is more optimistic, though I confess he does not support his optimism with encouraging arguments. All the same, he is right to be optimistic. The ultimate treason to mankind is to lose confidence in humanity. □

Lord Ashby is a Fellow of Clare College, Cambridge and Chancellor of the Queen's University, Belfast, Northern Ireland.

Photoemission from surfaces

Photoemission and the Electronic Properties of Surfaces. Edited by B. Feuerbacher, B. Fitton and R. F. Willis. Pp. 540. (Wiley: Chichester and New York, 1978.) £19.50.

THE study of photoemission from solids has grown enormously in the past decade. Although the photoelectric effect has been known for many years and provided a key result for the development of quantum theory, its application to the study of electronic structure of solids is much more recent and the appreciation of its essential surface sensitivity has encouraged its inclusion in the growing armoury of techniques of surface science. Indeed, in the case of ultraviolet photoelectron

spectroscopy in particular, the degree and nature of its surface sensitivity is still an issue of considerable interest. The extent of this development is mirrored by the size of this book, a collection of 17 separate articles on the theory and experimental results of the technique, together with two articles on the intimately related problem of the electronic structure of clean surfaces and adsorbates on surfaces.

The editors have collected together a formidable team of authors for this work; very few of the 'big names' in the subject are missing and the resulting articles are authoritative and thorough. Of course, in any multi-author book, particularly with authors active in an expanding and developing research field, the treatment of the subject is not always even and overlaps do exist, particularly in the introduction of the basic ideas behind several articles. Frequently, however, this overlap is beneficial; the reader is presented with contrasting views of the same material and can emerge with a more balanced appreciation as a result.

Biology of RNAs

The Ribonucleic Acids. (Second edition.) Edited by P. R. Stewart and D. S. Letham. Pp. 374. (Springer: New York, Heidelberg and Berlin, 1978.) DM47.40; \$23.70.

As an antidote to the new-style undergraduate textbooks, awash with coloured diagrams and divided into easily digested segments, *The Ribonucleic Acids* is recommended to students. Here the content is solid, the illustrations mainly restricted to structural formulae in sombre black and white.

This book is intended for students who already have basic training in biochemistry, and, although dealing with a specialist subject, does not aim to be either fully comprehensive or overly detailed. The first chapter is, typically, an historical introduction by the editors and is followed by chapters dealing with different species of RNA—nuclear, transfer, messenger, and so on—and their transcription and translation. The final chapter is a collection of recipes for the preparation and fractionation of RNA, curious in a textbook designed for students but undoubtedly of value to research workers.

Although the chapters have been written by different authors, some attempt has been made to make the style and structure uniform. Reading of the interaction between the 3' end

An intrinsic problem of any book in a very active research field is one of timing; has the subject developed sufficiently to warrant a book or will it be rapidly dated by new developments? In this case the timing seems to be a good compromise. Certainly new developments are occurring, and will continue to occur, which may change the detailed interpretation of specific problems. Also, the construction of many new synchrotron radiation sources around the world is bound to cause a shift in emphasis to the increased use of photon energy and polarisation-dependent effects as a means of studying surfaces. On the other hand, the stress in many articles in this volume is on basic physical principles and as such the book is likely to prove valuable for several years to those involved in, or wishing to learn more about, photoemission from surfaces and their electronic structure.

D. P. Woodruff

D. P. Woodruff is Lecturer in Physics, University of Warwick, UK.

of 16S ribosomal RNA and the 5' end of mRNA in four different chapters suggests that a certain uniformity in content was also required! The balance between cataloguing the facts and critical appraisal of experimental results varies in different chapters, although perhaps some RNAs—nuclear RNA, for example—elicit a more controversial literature.

Distinguishing the characteristics of prokaryotes and eukaryotes is very much easier for research workers, where it is a case of 'us and them', than it is for students, who have no previous affiliations. The latter group of readers will find this book adds to their problems. Sometimes prokaryotes and eukaryotes are allocated different headings, sometimes they are contrasted in the same sentence. This makes the text easier to read and less repetitious, but will undoubtedly perpetuate the existence of that interesting ribosome, found only in undergraduate essays, having 30S and 50S subunits containing 18S and 28S RNAs.

The references accompanying each chapter are extensive, reviews being listed separately. This information, together with the up-to-date overview of the biology of RNA that the text provides, should make this a useful book for research workers in related fields as well as the students for whom the book was written.

Pamela Hamlyn

Pamela Hamlyn is at the MRC Laboratory of Molecular Biology, Cambridge, UK.

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announcements

Awards

The following awards have been made by the Royal Society: the Copley Medal to **Professor R. B. Woodward** (Harvard University) for his contributions to experimental and theoretical organic chemistry; the Rumford Medal to **Sir George Porter** (Royal Institution) for his work on flash photolysis; the Davy Medal to **Professor A. Eschenmoser** (Swiss Federal Institute of Technology) for his work on synthetic organic chemistry; the Darwin Medal to **Dr G. Pontecorvo** (Imperial Cancer Research Fund) for his advances in genetics utilising lower organisms; the Hughes Medal to **Professor W. Cochran** (University of Edinburgh) for his advances in crystallography and lattice dynamics; the Leverhulme Medal to **Sir Frederick Warner** (Cremer and Warner) for his contributions to the developments of chemical and allied processes on the industrial scale; The Royal Society Eso Award to **A. G. Frame, J. M. Laithwaite, A. D. Evans, Dr J. F. W. Bishop and Dr T. N. Marsham** for their contributions to the development of the prototype fast reactor at Dounreay; the Royal Society Mullard award to **Dr J. W. Black** for his work on the development and discovery of two new types of drugs.

The 1978 ICE Diagnostics Techniques prize winners are **L. M. Callow and K. Hladky** of UMIST for their work on an electrochemical impedance corrosion monitor. The prize of £1,000 is to be awarded annually for five years and is to be administered by the Institution of Chemical Engineers. Entries for the 1979 award are now invited: ICE, 165-171 Railway Terrace, Rugby.

The following have been awarded the Indian National Science Academy's Medals for Young Scientists for the year 1978: **Dr Praveen Chaddah; Dr Renu Khanna Chopra; Dr K. C. Das; Dr A. R. Datta; Dr Vinay V. Deodhar; Shri S. Easwaramoorthy; Dr Chunilal Ghosh; Dr Jitendra Nath Goswami; Dr S. Ramasesha; Dr H. A. Ranganath; Dr Kalidas Sen.**

The Institution of Nuclear Engineers has awarded the first Hinton Medal to **Anthony R. W. Lunt** for his part in the engineering of the Dounreay prototype fast reactor.

The Beilby Medal and Prize for 1978 has been awarded by the RIC, the SCI and the Metals Society to **Dr John Christopher Scully** for his work in corrosion science in particular on stress-corrosion cracking. The award is made to British investigators (normally under the age of 40) for original work in chemical engineering, fuel technology or metallurgy. Applications for the 1979 award should be made to: The Sir George Beilby Memorial Fund, The RIC, 30 Russell Square, London WC1.

The trustees of the Lady Tata Memorial Trust for research in leukaemia have made the following international awards for the academic year 1978-79: from Great Britain—**H. Bayani; A. Karpas and H. Jacobs**; from France—**J. Grosslerner**; from Portugal—**E. Cardoso**; from Spain—**R. de N. Bastos**.

The Harrison Memorial Prize will be awarded to a British subject under the age of 30 years who has conducted the most promising original investigations in chemistry and published the results in a scientific periodical. Applications to the President of the Chemical Society, Burlington House, Piccadilly, London W1, by 31 December 1978.

The sixth of the Henry R. Worthington Technical Award for a prize of 10,000 dollars held under the auspices of the EEC is to be launched on a European-wide basis. It is to be awarded to young researchers from the universities and industry, working in the field of applied mechanics for the movement of fluids and for energy conversion systems. The contest regulations are available from Award Secretariat, Via Pirelli, 19-201234 Milan, Italy.

Meetings

2-3 October, **Workshop on Placenta—the Neglected Experimental Animal**, London (Dr Peter Beaconsfield, SCIP Research Unit, Bedford College, University of London NW1, UK).

2-4 October, **Symposium on Comparative Pathology of Zoo Animals**, Washington (Dr Montali, National Zoological Park, Washington, D.C. 20008).

2-5 October, **Clean Air Ways and Means**, Brighton (National Society for Clean Air, 163 North St, Brighton, Sussex, UK).

3-6 October, **Wind Energy Systems**, Amsterdam (2nd ISWES, BHRA Fluid Engineering, Cranfield, Bedford, UK).

3-6 October, **Postsynthetic Alterations of Information Molecules**, Lunteren (Miss Elly Looy, Institute of Molecular Biology, Padualaan 8, Utrecht, The Netherlands).

3-7 October, **Nuclex 78**, Basle (Congress Service of the Swiss Industries Fair, PO Box CH-4021 Basle, Switzerland).

4-6 October, **National Conference of Standards Laboratories**, Gaithersburg (Brian Belanger, A345 Physics Building, NBS, Washington, D.C. 20234).

4-7 October, **3rd European Symposium on Hormones and Cell Regulation**, Bischenberg (H. J. van der Molen, Dept of Biochemistry II, Medical Faculty, Erasmus University, PO Box 1738, Rotterdam, The Netherlands).

5-6 October, **Carliogenecity of Foods**, Chicago (Dr John J. Hefferren, ADA Health Foundation Research Institute, 211 East Chicago Ave, Chicago, Illinois 60611).

5-6 October, **Future Trends in Clinical and Laboratory Research in Cancer**, Paris (Dr W. H. Fridman, IRSC, BP No. 8, 94800 Villejuif, France).

5-6 October, **International Liquid Chromatography Symposium: Biological/Biomedical Applications**, Boston (Gerald L. Hawk, International Division, Waters Associates, Inc. Milford, Massachusetts 01757).

6-1 October, **Annual Meeting of the Swiss Society of Mineralogy and Petrography**, Brigue (Prof. M. Grönenfelder, Institut de Cristallographie et Petrographie ETH, 8092 Zurich, Switzerland).

9-12 October, **4th International Congress for the Study of Bauxites, Alumina and Aluminium**, Athens (Prof. S. S. Augustithis, National Technical University, Dept of Mineralogy, Petrology and Geography, PO Box 1006, Athens, Greece).

9-13 October, **1st International Congress on Bentonites**, Sassari (Istituto di Mineralogia e Geologia, Facoltà di Agraria, Università de Sassari, Italy).

9-19 October, **Atmospheric Sensing with Lasers**, London (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

10-12 October, **3rd Annual Conference on Materials for Coal Conversion and Utilization**, Gaithersburg (Bernard Levin, B64, Technology Building, NBS, Washington, D.C. 20234).

10 October, **Satellite Doppler Tracking and Geodetic Applications**, London (The Royal Society, 6 Carlton House Terrace, London SW1, UK).

10-13 October, **4th European Electro-optics Conference and Exhibition**, Utrecht (Carole Meads, Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

10-14 October, **International Symposium on Interaction of Marine Geodesy and Ocean Dynamics**, Miami (Dr George A. Maul, NOAA-AOML, 15 Rickenbacker Causeway, Miami, Florida 33149).

11 October, **Carcinogenic Risks from Food—Real or Imaginary?** London (SCI Food Group, 14 Belgrave Square, London SW1, UK).

12-14 October, **International Symposium on Pituitary Microadenomas**, Milan (Secretariat, Clinica Neurochirurgica dell'Università, Ospedale Policlinico, Pad. Beretta, via F. Sforza 35, 20122 Milan, Italy).

12-18 October, **1st Ibero American Congress of Cell Biology**, Mendoza (Secretariat, Casilla de Correo 56,5500 Mendoza, Argentina).

12-18 October, **4th Latin American Congress of Electron Microscopy**, Mendoza (Secretariat, Casilla de Correo 56,5500, Mendoza, Argentina).

Reports & Publications

UK & Ireland—August

Directorate of Ancient Monuments and Historic Buildings. The Scientific Treatment of Material from Rescue Excavations. (A Report by a Working Party of the Committee for Rescue Archaeology of the Ancient Monuments Board of England.) Pp.28. (London: Directorate of Ancient Monuments and Historic Buildings, DOE, Fortress House, Savile Row, 1978.) [18]

Centre for Agricultural Strategy. Strategy for the UK Dairy Industry. (CAS Report 4.) Pp.186. (Reading: Centre for Agricultural Strategy, The University, 1978.) £2.95. [18]

Bulletin of the Geological Survey of Great Britain. No. 66: The Pleistocene Succession at Kenn, Somerset. By D. D. Gilbertson and A. B. Hawkins. Pp. iv+41. (London: HMSO, 1978.) £3.75 net. [18]

Central Electricity Generating Board. Renewable Sources of Energy: The Prospects for Electricity. By Glyn England. Pp. 11. (London: CEGB, Publicity Office, 15 Newgate Street, EC1, 1978.) [38]

Scottish Plant Breeding Station. Fifty-seventh Annual Report, 1977/1978. Pp. 148. (Pentlandsfield, Roslin, Midlothian: Scottish Society for Research in Plant Breeding, 1978.) [38]

The British Industrial Biological Research Association. Annual Report 1977. Pp. 55. (Carshalton, Surrey: The British Industrial Biological Research Association, 1978.) [38]

Project Daedalus: The Final Report on the British Interplanetary Society Starship Study. Edited by Dr. A. R. Martin. (Journal of the British Interplanetary Society Supplement.) Pp. 192. (London: British Interplanetary Society, 12 Bessborough Gardens, SW1, 1978.) £6; \$12 post free. [48]

Department of Industry. Research and Development Requirements and Programmes Report 1977-78. Pp. 44. (London: Department of Industry, Technology Library, Abell House, John Islip Street, SW1, 1978.) gratis. [48]

The Importance of Being Curious. By Professor Hans L. Kornberg, FRS. (The Third Leverhulme Memorial Lecture.) Pp. 26. (Liverpool: Liverpool University Press, 1978.) [118]

Departments of the Environment and Transport. Report on Research and Development 1977. Pp. 66. (London: HMSO, 1978.) £2.25 net. [118]

Journal of Petroleum Geology, Vol. 1, No. 1, July 1978. Pp. 1-122. Published quarterly. Subscriptions: £20 for one year, UK only, post-free, by surface mail; \$US40 to all other countries, post-free, surface mail. (Beaconsfield, Bucks: Scientific Press, Ltd., 1978.) [148]

National Institute of Agricultural Botany. Fifty-Eighth Report and Accounts, 1977. Pp. 112. (Cambridge: National Institute of Agricultural Botany, Huntingdon Road, 1978.) [148]

Progress in Perspective: a View of Bayer. By René Cuitforth. Pp. 48. (Richmond, Surrey: Bayer UK, Ltd., Bayer House, 1978.) [168]

Department of Energy. Digest of United Kingdom Energy Statistics 1978. Pp. vii+138. (A Publication of the Government Statistical Service.) (London: HMSO, 1978.) £7.50 net. [168]

Seawatching. By Tony Soper and Noel Cusa. Pp. 32. (Over, Cambridge: Dinosaur Publications, Ltd., 1978. Published for the National Trust.) 60p. [188]

Smith Kline and French Foundation: Smith Kline Foundation. Annual Report 1977-9. (Welwyn Garden City, Herts.: Smith Kline and French Foundation, 1978.) [188]

Threshold Limit Values for 1977. (Guidance Note EH 15/77 from the Health and Safety Executive) Pp. 20. (London: HMSO, 1978) 30p. [188]

John Innes Institute. Sixty Eighth Annual Report, 1977. Pp. 183. (Norwich: John Innes Institute, 1978.) £2.50. [218]

Office of Population Censuses and Surveys. Mortality Statistics: Accidents and Violence. (Review of the Registrar General on Deaths Attributed to Accidental and Violent Causes in England and Wales, 1976.) Pp. viii+39. (London: HMSO, 1978.) £1.50 net. [258]

Where Were You, Brother?: An Account of Trade Union Imperialism. By Don Thomson and Rodney Larson. Pp. 138. (London: War on Want, 467 Caledonian Road, N7, 1978.) £1.20. [298]

Council for National Academic Awards. Annual Report for 1977. Pp. 75. (London: Council for National Academic Awards, 344/354 Gray's Inn Road, WC1, 1978.) [298]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 289, No. 1365: The Origin of the Triassic Clay Assemblages of Europe with special reference to the Keuper Marl and Rhaetic of Parts of England. By C. V. Jeans. Pp. 549-639+plates 1-5, pullout 1. UK £13.25; Overseas £13.75. Vol. 290, No. 1366: Sea Floor Development—Moving Into Deep Water. A Discussion organized by Sir Angus Paton, F.R.S., Sir Peter Kent, F.R.S., Sir George Deacon, F.R.S., Sir Kenneth Hutchison, F.R.S., and M. B. F. Ranken, in collaboration with the British National Committee on Ocean Engineering of the Council of Engineering Institutions. Pp. 1-189+plates 1-7; two pullouts. (London: The Royal Society, 1978.) [318]

Chemical Industry Contacts Directory for Journalists. Pp. 17. (London: Chemical Industries Association, 93 Albert Embankment, SE1, 1978.) [318]

Other countries—August

Safe Disposal of High Level Nuclear Reactor Wastes: a New Strategy. By A. E. Ringwood. Pp. vii+63. (Canberra: Australian National University Press, 1978.) [18]

Government of India: Department of Space. 1977/78 Annual Report. Pp. (Bangalore: ISRO HQ, Janardhan Tower, Residency Road, 1978.) [18]

Bulletin of the American Museum of Natural History. Vol. 160, Article 3: Social Signals of Adult American Alligators. By Leslie D. Garrick, Jeffrey W. Lang, and Harold A. Herzog, Jr. Pp. 153-192. (New York: American Museum of Natural History, 1978.) \$2.95. [18]

Australian Journal of Zoology. Supplementary Series No. 61: The Australian Social Wasps (Hymenoptera: Vespidae). By O. W. Richards. Pp. 132. (East Melbourne: Editor-in-Chief, Editorial and Publications Service, CSIRO, 314 Albert Street, 1978.) [28]

United States Department of the Interior: Geological Survey. Bulletin 1448: Geology of the Juazohn Quadrangle, Liberia. By Russell G. Tysdal. Pp. iv+39. (Washington, DC: US Government Printing Office, 1978.) [28]

The Kroc Foundation. Medical Research Programs, 1977. Pp. 134. (Santa Ynez, California: The Kroc Foundation, 1978.) [28]

Commissariat à l'Energie Atomique. Rapport Annuel 1977. Pp. 119. (Paris Cedex: Commissariat à l'Energie Atomique, 31-33 Rue de la Federation, 1978.) [38]

Smithsonian Contributions to Zoology. No. 272: Further Observations on *Oratosquilla*, with Accounts of Two New Genera and Nine New Species (Crustacea: Stomatopoda: Squillidae). By Raymond B. Manning. Pp. iii+44. (Washington, DC: Smithsonian Institution Press, 1978. For sale by US Government Printing Office.) [48]

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